

1 *Review*
2 **Pangolin Footprints: Infectivity, Virulence and Long**
3 **COVID**

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Abstract: This review focuses on three closely related models that use protein intrinsic disorder to link the N and M to infectivity, virulence and, potentially, long COVID. While the models, Shell Disorder Models (SDMs), were initially created using computational and empirical molecular techniques based on protein intrinsic disorder, experimental and clinical data were continuously used for refinement and checked for reproducibility and reliability of SDMs. Interestingly, SDMs are uniquely able to link the potentially major cause of infectivity, virulence and long COVID under one coherent concept of protein intrinsic disorder. An example of this is SDMs' ability to provide a novel and coherent explanation for the differences in the virulence and infectivity of Omicron, SARS-CoV-1 and non-Omicron SARS-CoV-2. SARS-CoV-2 has been clinically shown to induce much greater shedding of infectious particles in patients. Curiously, all known SARS-CoV-2-related viruses, excluding SARS-CoV-1, have an abnormally hard outer shell (low M disorder), which is associated with burrowing animals, such as rabbits and pangolins. Evidence of a unique molecular and evolutionary relationship ("pangolin footprints") between pangolins and COVID-19 is examined. SDMs suggest that this hard outer shell is responsible for the high infectivity of COVID-19 and , potentially, long COVID, as the hard M provides resistance to the antimicrobial enzymes found in the immune and respiratory systems. The implications could provide clues towards further research involving long COVID and infectivity, including possible reservoirs among macrophages. This also explains clinical observations of the persistence of the virus throughout the body months after infection. In the case of virulence, greater disorder in N contributes to more rapid replication by providing more efficient protein-protein binding. As a results, N disorder correlates with viral titer and therefore virulence and, to some extent, infectivity.

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Keywords: coronavirus; COVID; intrinsic disorder; membrane; nucleocapsid; nucleoprotein; Omicron; pangolin; shell; virulence; long COVID; attenuation; variant; immune; perforin; complement system; macrophage; reservoir; Artificial Intelligence; AI; hard shell; lysosome; unstructured; NL63; spike;

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Abbreviations:

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COVID: Coronavirus Disease; CoV: Coronavirus; SARS: Severe Acute Respiratory Syndrome; HCoV: Human coronavirus. SDM: Shell Disorder Model; POND[®]-VLXT: Predictor of Natural Disordered Regions using VLXT; PID: Percentage of Intrinsic Disorder (number of disordered residues divided by the total number of residues); Pang2017: SARS-CoV-2 related pangolin-CoV isolated in 2017; Pang2019: Pangolin-CoV isolated in 2019, N: Nucleocapsid protein, M Membrane protein; S: Spike protein; SARS-CoV-2, BANAL: SARS-CoV-2 related Bat-CoVs found in Laos, NL63: A common HCoV; RaTG13: A SARS-CoV-2 related bat-CoV discovered in Yunnan; AI: Artificial intelligence; EBOV: Ebola virus; NiV: Nipah virus; DENV: Dengue virus; HIV: Human immunodeficiency virus; YFV: Yellow fever virus; ZIKV: Zika virus;

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1. Introduction

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1.1 Overview

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This review focuses on the three closely related models as related to COVID-19 (Coronavirus Disease 2019) [1-3]. The models use protein intrinsic disorder to link the N and M to infectivity, virulence and, potentially, long COVID. While the three models, Shell Disorder Models (SDMs) [4], were computationally created using empirical

molecular techniques based on protein intrinsic disorder, experimental and clinical data will be examined for the reproducibility and reliability of SDMs. Interestingly, SDMs are uniquely able to link the potentially major cause of infectivity, virulence and long COVID under one coherent concept of protein intrinsic disorder. An example of this is SDMs. For instance, it is able to provide a novel and coherent explanation for the differences in virulence and infectivity between SARS-CoV-1 and SARS-CoV-2, which has been clinically shown to induce much greater shedding of infectious particles in patients. Curiously, all known SARS-CoV-2-related viruses, excluding SARS-CoV-1, have an abnormally hard outer shell (low M disorder), which is associated with burrowing animals, such as rabbits and pangolins. Evidence of a unique molecular and evolutionary relationship ("pangolin footprints") between pangolins and COVID-19 is examined. SDMs suggests that this hard outer shell is responsible for the high infectivity of COVID-19 and, potentially, long COVID, as the hard M provides resistance to the antimicrobial enzymes found in the immune and respiratory systems. The implications could provide clues towards further research involving long COVID and infectivity, including the search for possible reservoirs among macrophages. This also explains clinical observations of the persistence of the virus throughout the body months after infection. In the case of virulence, greater disorder in N contributes to more rapid replication by providing more efficient protein-protein binding. As a results, N disorder correlates with viral titer and therefore virulence and, to a limited extent, infectivity. As S (spike protein) is currently yet to be part of SDMs, S is mentioned sparingly as part of a discussion involving the potentials and limitations of SDMs. It should also be reminded that while there are current review and research papers that attempt to link various viral proteins to virulence or infectivity [5-8], we will attempt to show that the approach using protein intrinsic disorder provides a more coherent concept that links virulence and infectivity via M and N. S could also be later added to the framework at some point.

1.2 Pangolin Footprints: Enigmas of COVID-19 Related Viruses

It has been more than 4 years since the first outbreak of COVID-19 [1-3]. Even as we are just beginning to understand what was previously unclear about it, there is still much to be uncovered and resolved. For instance, why is SARS-CoV-2 highly contagious in contrast to 2003 SARS-CoV (SARS-CoV-1, SARS: Severe Acute Respiratory Syndrome, CoV: Coronavirus)? The total number of people infected thus far is more than 700 million [3], whereas there were only about 10,000 cases in the 2002-3 SARS outbreak [4]. Why is SARS-Co-2 less virulent and much more infectious than SARS-CoV-1? What are the structural differences responsible for the differences? What is the cause of long COVID? Early in the outbreak it was believed that the reason for the extraordinary contagiousness of COVID-19 lies solely on the Spike (S) protein [9-12]. As more computational, clinical, and experimental data became available, the reproducibility of such a hypothesis can be increasingly questioned even though there are few current papers that examine this issue especially pertaining links between infectivity and virulence in a coherent manner, which we believed is best approached using the concept of protein intrinsic disorder. This paper re-examines the roles of two highly important, though less-researched, proteins: M and N, while keeping the functions of S in mind.

The framework of many of the studies were established using an AI tool to study the sequence of the M and N proteins. One important but peculiar discovery using the protein disorder AI tool, PONDR®-VLXT [13-17], is that all SARS-CoV-2-related viruses, not just SARS-CoV-2, have among the hardest outer shells (low M disorder) known within the CoV family [18-21]. It is believed that it is this anomaly pertaining to M that is primarily responsible for the high contagiousness of COVID-19, since a harder M provides greater resistance for the virus against the large array of antimicrobial enzymes present in the saliva and mucus [21-28] and, thus, making it more likely for the host to shed much more infectious particles [29]. N, on the other hand, could help modulate the infectivity and virulence as greater N disorder allows for more

efficient protein-protein/RNA/lipid binding [4,16,30-33] that could lead to more rapid replication of the virus, especially in vital organs [34-40].

A retrospective search after the initial COVID-19 outbreak yielded a bat-CoV sample (RaTG13) obtained from a Yunnan cave in 2013 that had a 96.4% genetic identity with SARS-CoV-2 [4-43]. Furthermore, two sets of pangolin-CoVs that were obtained from pangolins confiscated by customs in Guangxi (GX) and Guangdong (GD) provinces during 2017-18 (Pang2017, Pang2018) and 2019 (Pang2019) periods respectively have about 90% genetic proximity to SARS-CoV-2 [44-48]. Later, pangolin samples obtained in Vietnam showed similar results [49]. Likewise, a series of COVID-19 related bat-CoVs (BANAL) were found in Laos [50-51]. In fact, one of the samples, BANAL-52, had an even greater genetic proximity (96.8%) to SARS-CoV-2 than that of RaTG13 to SARS-CoV-2.

A search for similarly hard M yielded CoVs associated with burrowing animals such rabbits. This enigmatic association explains the true intimate relationship between all COVID-19 related viruses and pangolin-CoVs since pangolins are also burrowing animals [4,18-20,52-54]. The hard outer shell (M) is necessary since the virus has to be able to survive longer in buried feces before further transmission.

While most scientists believe that COVID-19 is the result of a zoonotic spillover, a better understanding of the evolution helps. The knowledge of N and M proteins can make the understanding of this evolution more complete as it bridges the relationship between SARS-CoV-2 and pangolin-CoV. The relationship as evidenced by the pangolin molecular "footprints" can account for many of the behaviors of COVID-19. The implications of this relationship will be re-visited in greater detail later.

One odd characteristic found in all SARS-CoV-2-related viruses and their variants found thus far is the unusually hard outer shell (low M disorder) that is typically found only in CoV associated with a burrowing animal. This hallmark is one of the "pangolin footprints" found in all COVID-19 related viruses discovered so far. The other set of footprints involve attenuations arising of lesser disorder in N found in Pang2017 and, later, in the variant Omicron [18-20,52-56]. The harder inner shell (N), especially in Pang2017, may be also a reflection of N protecting the viral RNA in buried feces, just like M [52,55].

Evidence of pangolin footprints offers clues that are beginning to uncover many of the mysteries of COVID-19 that are still haunting us. These include questions such as: why is SARS-CoV-2 much more infectious than SARS-CoV-1? Why is COVID-19 is highly infectious to this day? Why is SARS-CoV-2 less virulent than SARS-CoV-1? Why is Wuhan-Hu-1 much more virulent than Omicron? What is the actual structural cause of long COVID? Many of these questions have remained unanswered, but the study of the M and N proteins is now beginning to provide some answers from one integrated concept, namely the pangolin footprints. We should not be at all surprised by the explanatory prowess of the N and M proteins, as they are the most abundant proteins found in the cell and virion, respectively.

1.3 Long COVID

Yet another major mystery in COVID-19 is the presence of long COVID among many patients, i.e. the persistence of symptoms long after recovery [57-58]. It should also be noted that S, unlike M, is unable to account for the persistence of the virus even months after the initial infection as observed by clinical studies. Even as progress has been made in research to explain the mechanism and cause of long COVID, long COVID has remained by and large a mystery [59-64]. In this paper, we will review an alternative explanation that has already been previously mentioned. This explanation involves the unusually hard M found in SARS-CoV-2 that allows the virus to resist immune enzymes

and enables the virus to hide in macrophages. The abnormally hard outer shell, M, that protects the virion from the onslaught of the antimicrobial enzymes found in saliva and mucus [19-28,40,52], is also likely to reduce the chances of the elimination of viral particles via actions of the immune system. We will examine literature pertaining to current knowledge of immunology that could provide a potential framework for the mechanism of long COVID caused by hard M. SDMs will be used to gain further insight to the potential mechanisms involved.

The abnormally hard outer shell, M, that protects the virion from the onslaught of the antimicrobial enzymes found in saliva and mucus [19-28,40,52], is also likely to reduce the chances of the elimination of viral particles via actions of the immune system. Upon examination of immunological principles, Shell-Disorder Models (SDMs) even go on to pinpoint the exact immunological mechanisms that are likely to be hindered.

2. The Shell Disorder Models (SDMs): Three Closely Related Models

2.1 Three Closely Interrelated Models

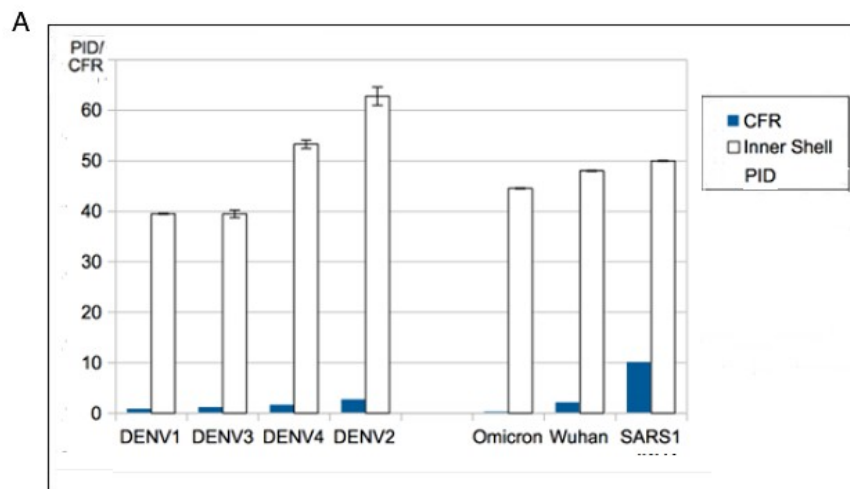
Three closely related models were developed using the concept of protein intrinsic disorder via AI tools. Protein intrinsic disorder refers to the lack of structure in an entire or part of a protein. Disorder is known to have various important functions especially in protein-protein/RNA/DNA/lipid/carbohydrate binding, and various tools have been developed to recognize disorder regions and disordered proteins. Among the first developed is PONDR®-VLXT, which involves the use of neural network AI to recognize disordered residues given the sequence input [13-17]. PONDR®-VLXT has been shown to be a highly appropriate tool particularly when it involves viral proteins of a large variety of viruses including Ebola virus (EBOV), Dengue virus (DENV), Nipah virus, and HIV [4,18-20,34-40,52,-56,65-67]. PONDR®-VLXT is especially suited for the study of viral structural proteins as it is highly sensitive in the detection of disorder in structured proteins [16]. The first SDM was initiated before 2008 when PONDR®-VLXT was used to examine the shell proteins of a variety of viruses. A useful number used is percentage of intrinsic disorder (PID), which is defined as the number of disordered residues predicted divided by the total number of residues in a protein chain [4,65]. A disordered residue is predicted to be disordered if its VLXT score is 0.5 or above. Upon comparison of the shell disorder of a fairly large number of viruses, it became obvious that the outer shell of HIV, especially HIV-1, has an abnormally high average disorder in its outer shells. It was also discovered that HSV and HCV share this similar characteristic, even though it is not as pronounced as in HIV-1 [418-20,34-40,65-67]. Since very few other viruses, if any, have this characteristic, it seems to have to do with the ability of the viruses to evade the immune system, and results in the absence of effective vaccines for the mentioned viruses. This SDM was labeled “Viral Shapeshifting” [4] and became the parent model for two other closely related SDM, as seen in Figure 1. Given the behaviors of HIV, HCV, and HSV, higher disorder at the outer shell can also be associated with the ability to penetrate hard-to-reach places such as the brain and placenta, as in the case of the Zika virus [34,35]. Protein disorder provides for greater efficiency in protein-protein/DNA/RNA/lipid/glycoprotein binding [16.30-33,55,58].

Table 1. The Three Shell Disorder Models (SDMs). SDMs were developed using principles of protein intrinsic disorder applied to viral shell proteins.

Year of First	Shell Disorder	Details
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Publication	Model	
2008	Viral Shapeshifter Model (Parent)	Disorder of shell proteins was measured for a wide variety of viruses. Only very few viruses have been found to have unusually high disorder at the outer shell (HIV-1, HCV, and HSV). There is no effective vaccine yet found for the three viruses.
2012	CoV Transmission SDM	Links between modes of transmission (fecal-oral and respiratory) and N-M disorder were found.
2015	Virulence-Inner Shell Disorder Model	Strong correlation between inner shell disorder and virulence of a wide variety of viruses including DENV, EBOV, NiV, and SARS-CoV-1/2.

Another model was developed and published in 2015 when it was discovered that there is a strong correlation between DENV virulence and disorder at the inner shell protein [34,35]. Similar correlations were found in a large variety of other viruses including NIV, CoVs, and EBOV [4,34-40,52-56,65-67]. The presence of such correlations has to do with the fact that the inner shells are often associated with replication in many viruses, and because disorder provides for greater efficiency in protein-protein/RNA/DNA/lipid/glycoprotein binding [16,30-33]. These form the basis of the Virulence-Inner Shell-Disorder Model (Table 1).



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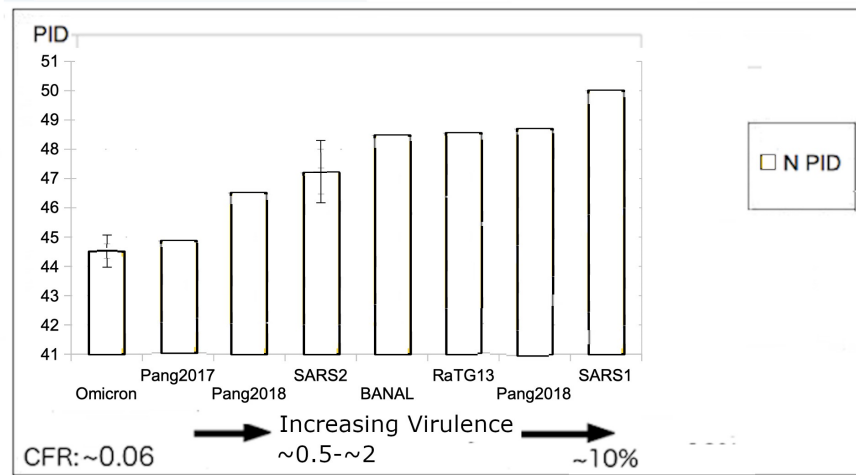


Figure 1. Virulence-Inner SDM (Shell Disorder Model). **A.** Shells PID of DENV and SARS-CoV-2. Inner shell of DENV has been found to be correlated to virulence ($r=0.95$) [34,35]. **B.** Correlation between the SARS-related viruses and N PID. The correlation is based on estimated CFRs of SARS-CoV-1/2 and Omicron. Note: DENV and SARS-CoV-1/2 shells are not correlated. They are just placed together for illustrative purpose only.

The third SDM was first published in 2012 [53] before MERS-CoV was discovered in 2013 [55]. The model divided CoVs into three groups, labeled A-C, that can be seen in Figure 2, with group D added during the COVID-19 pandemic [18-20,40,53,55,56]. Group D was not recognized in the initial model because there were very few CoVs that involved burrowing animals such as rabbits and pangolins at that time [4,18-16,52,53]. Before the COVID-19 pandemic, nearly all CoVs in available our database have M PIDs of at least 8% (See Figure 2 and Supplementary Table). The exceptions that have M PIDs lower than 8% are CoVs associated with burrowing animals. It was during the pandemic that it was discovered that all SARS-CoV-2-related viruses have M PIDs lower than 7% (4-6.3%) (See Figure 2 and Table 2). While the statistical differences seem small, in reality, it is actually not anything small or trivial as we will see, later, that M is the most abundant protein that encases the entire virion. Even small changes will affect the rigidity of the entire shell. This CoV-Transmission SDM invokes the same molecular principle that the Virulence-Inner SDM uses, which involves greater disorder at the inner shell that provides greater efficiency in viral replication. However, the CoV-Transmission SDM extends the principle to the levels of fecal-oral and respiratory transmission potentials, by showing that respiratory transmission is viable only when sufficient copies of the virus are shed nasally. This results in N being adequately disordered. Multivariate analysis have found strong correlation between modes of transmission and levels of M/N PIDs with statistical significance (Multivariate analysis: $p < 0.001, r \sim 0.8, N = 32$, see **Suppl. Table** for further information).

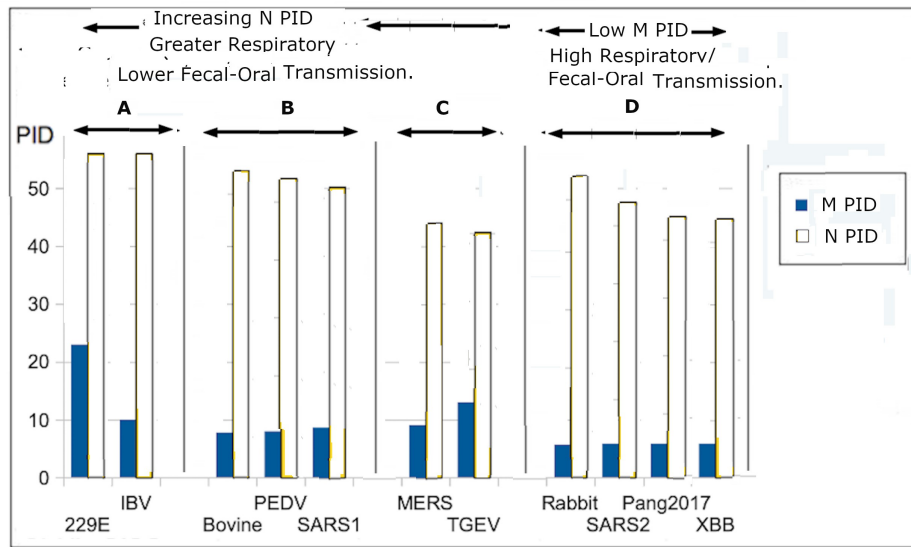


Figure 2. CoV Transmission Shell Disorder Model (SDM) CoVs. In groups A-C, the levels of respiratory/fecal-oral transmission are heavily dependent on N PID, whereas those in group D have unusually low M PIDs (M disorder) that are usually associated with burrowing animals such as pangolins. Group D includes all COVID-19-related viruses. (Multivariate analysis: $p < 0.001, r \sim 0.8, N = 32$, see **Suppl. Table** for further information)

The CoV-Transmission SDM categories SARS-CoV-1 in group B consist of CoVs with intermediate fecal-oral and respiratory transmission potentials. Upon the publication of the original paper, the MERS-CoV outbreak took place in 2012-13 when the SDM had to place MERS-CoV in group C, in which the CoVs have higher and lower fecal-oral and respiratory transmission potentials respectively [54]. This prediction has been reproduced clinically and experimentally [4]. It is also known that MERS-CoV has long been entrenched among camels, especially farmed camels, where it spreads easily by fecal-oral means.

3. Pangolin Footprints: Applying SDMs to SARS-CoV-2

3.1.. The First Sign of a Pangolin Footprint: Abnormally Hard Shell in All SARS-CoV-2-Related Viruses

When the CoV-Transmission SDM was first developed and implemented in 2012-13, strong correlation was seen between N disorder (N PID) and the modes of transmission [4,53,54]. However, statistical calculations detected a small correlation between M disorder (M PID) and modes of transmission. PID

is defined as the number of disordered residues divided by the total number of residues in the protein. At that time, it was not understood why such a correlation existed. It was not until the arrival of the COVID-19 pandemic that came with a torrent of data that things began to fall in place. The CoV-Transmission SDM was applied to the Wuhan-Hu-1 as soon as the M and N proteins became available, with Wuhan-Hu-1 having N and M PIDs of 48.2% and 5.9% respectively [14,40,52-55]. Using the original model, SDM placed SARS-CoV-2 in group B, which is the same group that SARS-CoV-1 is in.

SDM did detect something highly unusual about this virus that was seldom seen in our curated database of known CoVs: the outer shell of SARS-CoV-2 is abnormally hard, i.e. has a low M PID [18-20]. As more data became available, it became clear that the odd hard outer shell was not just something that pertains only to SARS-CoV-2 but to all COVID-19-related viruses, as seen in Table 2 and Figure 2. We can see that the hard M (low M PID < 7%, Table 2) extends to all SARS-CoV-2-related viruses, including pangolin-CoVs and bat-CoVs such as RaTG13 and the Laotian bat-CoV (BANAL). Details found in the tables can also be found in previous papers [52,55-56] and the protein sequences were obtained either from UniProt [69] or NCBI-Protein [70], and the PONDR®-VLXT scores were obtained by inputting the sequences into PONDR®-VLXT [13-17]. The PIDs (percentages of intrinsic disorder) were calculated as the number of disordered residues divided by the total number of protein in a protein chain [4,65-67].

Table 2. N/M PIDs and Sequence Similarities of COVID-19 Related Viruses CoVs with SARS-CoV-1 and Non-SARS-CoV-2-related Bat-CoVs as references. It should be noted that BA1 was the initial Omicron. **At least two Delta subvariants have been detected by SDMs.**

Coronavirus	Sequence Similarity M (%)	M PID (%)	Accession: UniProt (U); GenBank (G)	Sequence Similarity N (%)	N PID (%)	Accession: UniProt (U); GenBank (G)
SARS-CoV-1	90.5	8.6	P59596(U)	90.5	50.2	P59595(U)
Civet-SARS-CoV	90.1	8.6	Q3ZTE9(U)	90.01	49.1	Q3ZTE4(U)
Laotian Bat-CoV	-	6.0±0.2	-	-	48.3±0.2	-
[Banal-52]	98.7	6.3	UAY13220.1	99.3	48.2	UAY13225.1
[Banal-103]	98.7	5.9	UAY13232.1	99.1	48.5	UAY13257.1
[Banal-236]	99.1	4.1	UAY13256.1	99.3	48.5	UAY1326.1
Pangolin-CoV	-	5.6±0.9	-	-	46.6±1.6	-
2019	98.2	6.3	QIG55948(G)	98	48.7	QIG55953(G)
2018	97.7	4.5	QIQ54051(G)	93.8	46.3	QIQ54056(G)
2017	98.2	5.9	QIA48617(G)	94	44.9	QIA48630(G)
				93.32	46.5	QIA48656(G)
SARS-CoV-2						
Wuhan-Hu-1	100	5.9	YP009724393(G)	100	48.2	YP009724397(G)
Delta					47.1±0.5	
Delta1	99.1	5.9±0.01	QUX81285(G)		46.8	QYM89997(G)
Delta2	99.1	5.9	QUX81285(G)		47.5	QYM89845(G)
		5.9		99.1		-
Omicron	-		-		44.5±0.4	UFO692871(G)
Omicron BA1	98.7	5.7±0.4	UFO59282(G)	-	44.8	WIL50325
Omicron XBB	99.1	5.4	WBI50320(G)	98.6	44.2	

		5.9		98.2		
Bat-CoV		11.2±15			47.7±0.9	
RATG13	99.6	4.1	QHR63303(G)	99.1	48.5	QHR63308(G)
Bat 512	35.5	15.3	Q0Q463(U)	29.4	46.5	Q0Q462(U)
HKU3	91	7.7	Q3LZX9(U)	89.6	48	Q3LZX4(U)
HKU4	42.7	16.4	A3EXA0(U)	51.1	48.5	A3EXA1(U)
HKU5	44.7	11.8	A3EXD6(U)	47.9	47.1	A3EXD7(U)

It was only after the arrival of the pandemic, which came with new data, that we realized that group D has to be added, and there was a good reason why we missed group C in the beginning. A retrospective search for similarly low M PID can only be found in CoVs associated with burrowing animals such as rabbits [18-19,55]. Because there were very few such CoVs in the public database before the pandemic, the CoV-Transmission SDM was inevitably incomplete at that time. As according to our previously published papers [19,20], it also became obvious that the hard M was responsible the high transmissibility among humans by protecting the virus from the myriad of anti-microbial enzymes found in the mucus and saliva [18-20,40,45,52]. As a result, the host will shed large amounts of infectious particles nasally and orally. There is also reason to suspect that the abnormally hard M may have a lot to do with long COVID [40], as it is possible that the host immune system may often have difficulties destroying and getting rid of the viral particles.

3.2. Signs of Attenuation: Another Pangolin Footprint

The three SDMs present us with a set of tools that are able to create a wide range of specific predictions about SARS-CoV-2 and its closely related relatives. Just like the SDM's prediction was applied to MERS, the predictions have shown consistency when applied to experimental and clinical data. Figure 2 summarizes an example of the application of the Virulence-Inner SDM to COVID-19 that is consistent with clinical and experimental data. A strong correlation can be found between COVID-19 virulence and N PID. Using case fatality rate (CFR) as a benchmark for virulence, we are able to see that the CFR varies with the N disorder (N PID) with SARS-CoV-1 having the highest CFR of about 10%, followed by the non-Omicron SARS-CoV-2 variants, and then Omicron (Figure 1B). Even without using CFR, the Virulence-Inner SDM makes specific predictions about the levels of virulence of the SARS-related viruses and SARS-CoV-1. SARS-CoV-1 is predicted to be of higher virulence given its N PID of 50%, followed by Wuhan-Hu-1 (N PID: 48.2%), and the other non-Omicron variants (Delta N PID~47.1±0.5%). Omicron (N PID~44.5±0.4%) is the least virulent thus far [40,52,55-56]. It should also be known that CFR is just one measure of the link. As we shall see, it has been complemented with viral titer and cell/tissue studies. The SDMs are able to pinpoint the mechanisms that account for the predictions. In this case of COVID-19 virulence, the higher the N disorder, the greater the efficiency in viral replication that the virus is able to acquire, as N is intimately involved in the replication process and greater disorder provides for better protein-protein/RNA/DNA/Lipid/glycoprotein interactions.

3.3 Pangolin Footprints: Implications and Manifestations

Two important predictions seen in Figure 1B that have been reproduced are that Omicron and Pangolin-2017 (Pang2017) are attenuated based on their low N PIDs. This prediction was first mentioned in a publication in 2020 [14] and later reproduced in at least two laboratories. Omicron, however, did not emerge until November 2021. When the first Omicron sequence became available, it was found that the Omicron subvariant, BA1, has an N of 44.7%, which is very close to that of Pang2017 at 44.8% [20,52,55-56]. Just as Pang2017 was predicted to be attenuated. Omicron or, at least, BA1 has to be considered attenuated. It was later shown that all Omicron subvariants have similar N PID, with the later ones being even lower (XBB.1.16, N PID: 44.2%). Both Pang2017 and Omicron have been clinically and experimentally shown to be

attenuated with lower viral titer [40,51,74-79]. Interestingly, in contrast, Pang2019 N PID resembles that of non-Omicron variants, and, not coincidentally, its virulence and viral titers do not resemble those of Omicron or Pang2017 [80-82].

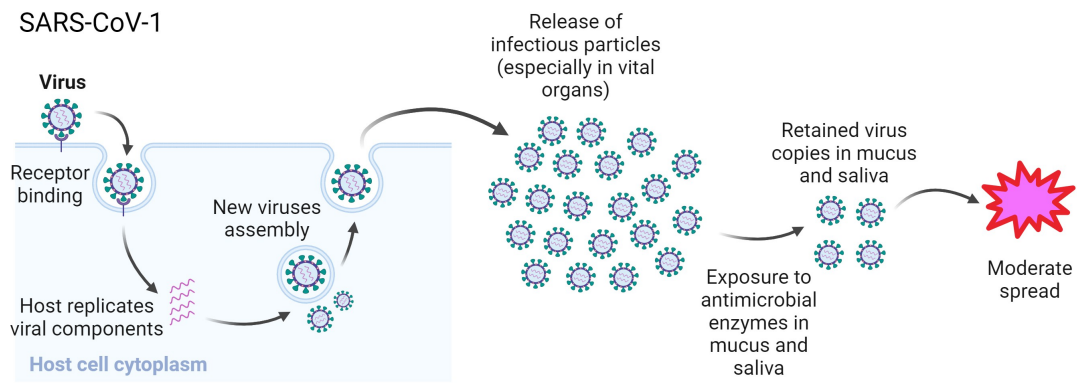
3.5. Implications and Manifestations: Infectivity and Virulence

Figure 3A summarizes the implications of SDMs and the effects of different N and M PIDs on the manifestation of COVID-19. Since SARS-CoV-1 (SARS1) has a higher N PID (~50%) and produces more viral particles, especially in the vital organs, but because its M is not as hard (M PID ~ 9%) [4], it offers less resistance to the onslaught of the salivary and mucosal antimicrobial enzymes and yet produces sufficient infectious particles for viable respiratory transmission. However, SARS-CoV-2 presents a different story. The Wuhan-Hu1 strain has a somewhat lesser N disorder (N PID ~ 48%) but a much lesser M disorder (M PID ~ 5.7%) [40]. According to SDMs, the virus will replicate well even in vital organs, but not as efficiently as SARS-CoV-1. Even though Wuhan-Hu-1 does not replicate in large quantities in contrast to SARS-CoV-1, the patient sheds more infectious particles because its harder M offers more resistance to the anti-microbial enzymes in the saliva and mucus [40,52]. For this reason, Wuhan-Hu-1 is virulent, but not as much as SARS-CoV-1. On the other hand, it is much more infectious than the latter, due to the immense amount of shedding. Omicron presents a different scenario with a similarly low M PID (~5.7%) but much lower N PID (~44.5%). With these, the SDMs predict low virulence but high infectivity, even if lower than Wuhan-Hu-1. All these predictions have been reproduced experimentally and clinically [52,55,71-82].

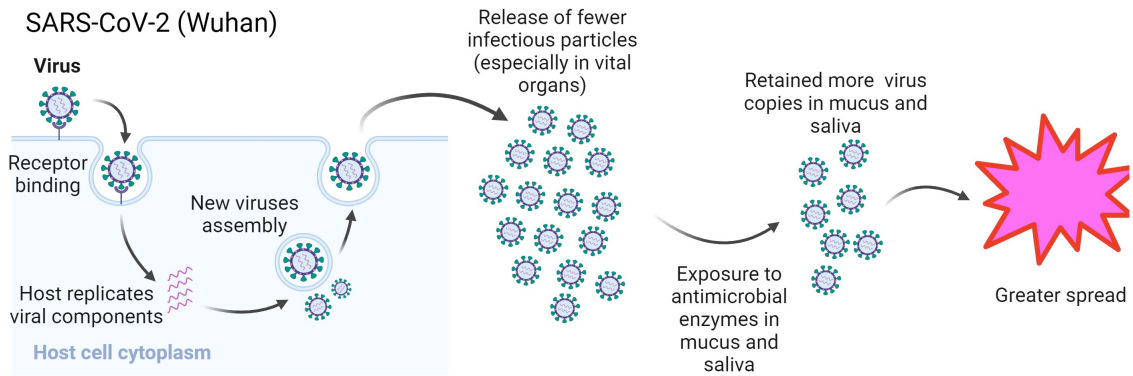
Figure 3A describes the underlying mechanism of how differences in N and M disorder affect manifestation of the virus, especially with respect to virulence and transmission. SARS-CoV-1 (SARS1) spreads efficiently via respiratory mode with greater virulence, but not as efficiently as SARS-CoV-2 (SARS2). SDMs attribute these properties to higher N and M PIDs (N PID: 50%, M PID: 9%) in contrast to those of SARS-CoV-2. Figure 3B illustrates the mechanism of COVID-19 attenuation via pangolins with analogy from Nipah virus [4,18,32,33,83].

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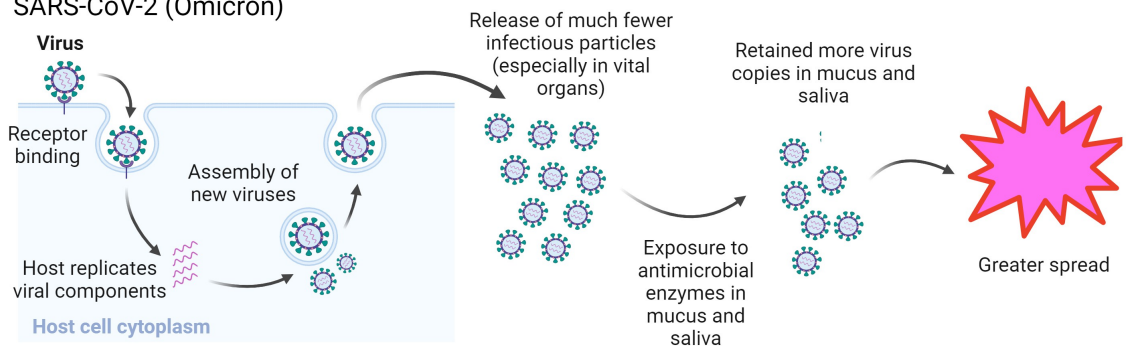
SARS-CoV-1



SARS-CoV-2 (Wuhan)



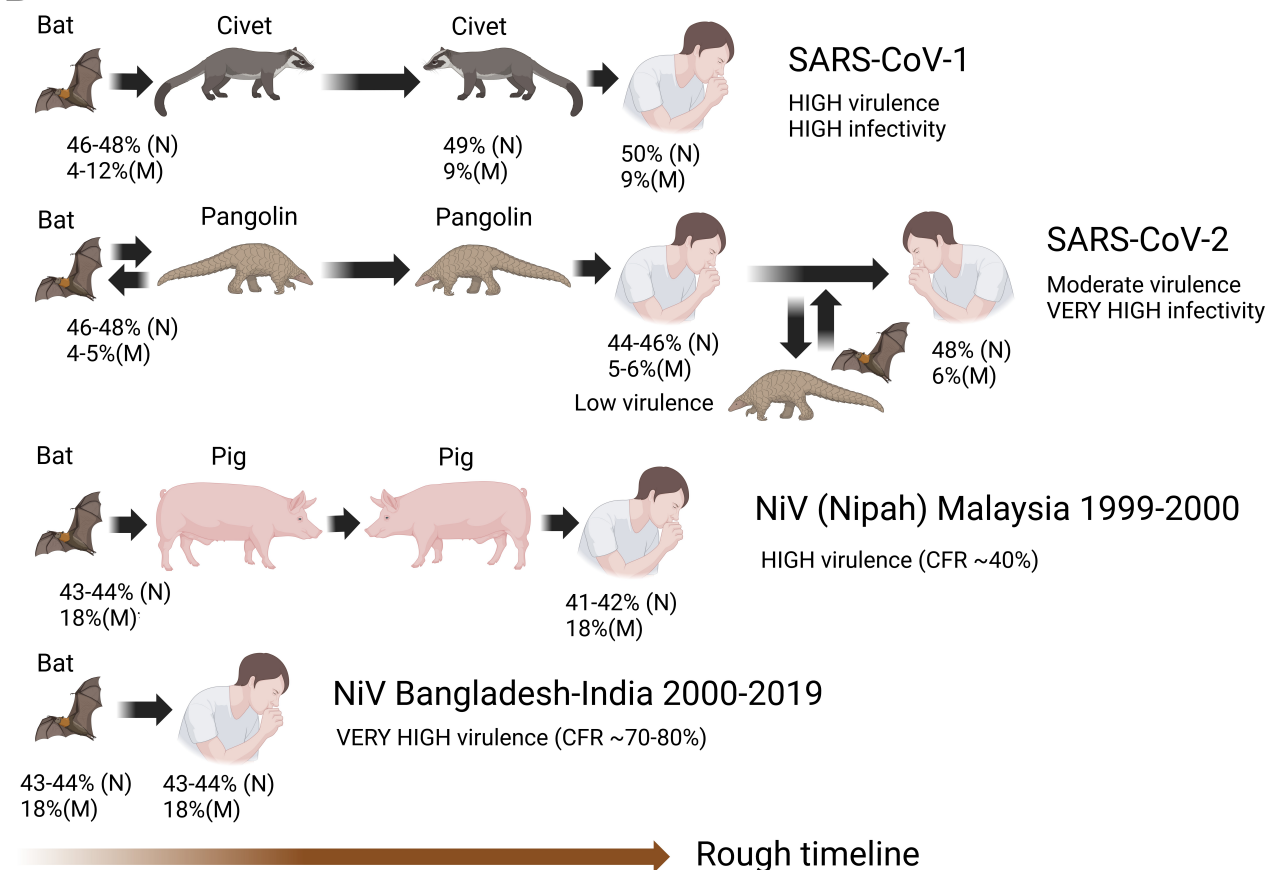
SARS-CoV-2 (Omicron)



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Figure 3. Effects of M and N disorder on virulence and infectivity. **A.** Effects of M and N disorder on virus replication. **B.** Effects of environment on virulence. SARS-CoV-1 produces high quantities of viral particles, especially in vital organs, because of its high N disorder, but by the time they reach the nasal and oral cavity, many have already been eliminated by mucosal and salivary antimicrobial enzymes as a result of its lower M PID. SARS-CoV-2, on the other hand, produces less particles in the entire respiratory system, but more particles are able to resist the antimicrobial enzymes because of its hard M, and, therefore, its patients shed more particles. NiV is highly virulent when it is spread directly from bats, whereas the variant that had pigs as an intermediate host was less virulent. The inner shell disorder of the latter was observed. Similar observations were made for SARS-CoV-1/2.

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While Figure 3A summarizes the mechanisms in which infectiousness and virulence is modulated, Figure 3B shows the evolutionary implications of the mechanisms described. The reason that SARS-CoV-1 was more virulent is likely the result of its shortness of time the virus was in its intermediary host, civet cat, before the zoonotic transmission to humans. In contrast, the Nipah virus (NiV) is usually highly virulent when the transmission to human is directly from bats, as in the cases seen in Bangladesh and India. Meanwhile, the 1999-2000 outbreak involved infection of humans via pigs as intermediary hosts, and the human CFR was about 50%, compared to the 75% CFR in the cases from India-Bangladesh [4,33,36,83].

The fact that it was discovered via PONDR®-VLXT that the disorder of inner shell proteins of the virus from the Malaysia outbreak was lower than those from the Bangladesh-India outbreaks is consistent with the prediction of the Virulence-Inner SDM and disorder observations of viruses from the same family. Attenuation of the virus came during its infection of pigs, in which fecal-oral transmission is the most convenient route in a farm environment. The hardening of the inner shell protein poses several advantages. Firstly, the fecal-oral route does not necessitate higher disorder in its inner shell protein and greater disorder requires greater energy, which is a waste if not utilized. Secondly, a harder inner shell offers some extra protection to the viral genome. Bat CoVs in general have higher N disorder, possibly arising from the need for certain levels of respiratory transmission potentials because of the behavior, involving flights, of bats. Respiratory diseases, such as avian influenza, have been known to spread between birds during flight [84-87]. There is therefore no reason not to believe that respiratory viruses such as CoVs can spread between bats during flights. SDMs support this notion when we see IBV, which is an avian coronavirus, has among the highest N PIDs and higher N PID is associated with higher respiratory transmission.(Figure 2). Indeed, as we will see later, bat-CoV N PID are usually higher than those of most other CoVs.

Given the generally lower N PID and, thus, virulence, it makes more sense if the precursor SARS-CoV-2 was deeply entrenched in an intermediary animal host. There are important reasons to believe that pangolins were involved. A smoking gun can be found in the abnormally hard M found in all COVID-19 related viruses that suggests a burrowing animal. The presence of SARS-CoV-2-related pangolin-CoVs are apparently widespread in Southern China and Southeast Asia, as evidenced by the discoveries of pangolin-CoVs in Guangxi, Guangdong, and Vietnam [42-49]. Figure 3B reminds us that pangolin is a likely reservoir, with Pangolin-CoVs moving to and fro among bats, humans, and pangolins.

3.6. Physiological Mechanisms Allow Dichotomy between Virulence and Infectivity: Mucociliary Clearance (MCC)

It has to be understood that the CoV-Transmission SDM revolves around the need to have sufficient concentration of infectious particles for the virus to have viable respiratory transmissibility. This can be facilitated by an abnormally hard M, more disorder in N, or both, since a harder M makes the virion more resistant to damage arising from the anti-microbial enzymes found in the mucus and saliva [21-28], whereas a more disordered N could induce more efficient viral replication as the result of more effective protein-protein binding. The latter is again used in the Virulence-Inner SDM, in which higher N disorder helps rapid replication of viral particles, especially in vital organs. How do the two factors interplay to allow us consistency among the various SDMs? A part of the answer has been explained in Figure 3A, which summarizes how virulence and infectiousness are intertwined. It does not, however, tell the whole story, especially with regard to how the physiology of the host helps in the entire process.

Knowledge of molecular physiology has allowed us to understand the roles that the respiratory system plays in the entire transmission process. Mucociliary Clearance (MCC) or Mucociliary Escalator is of particular interest [88-91]. It encompasses a body of knowledge that describes how the respiratory

system expels foreign bodies, including viruses and bacteria, via a mechanism known as MCC. MCC is a sophisticated network of hairy ciliated cells covered with mucus that transport foreign bodies away from the lungs towards upper respiratory regions to be shed, as seen in Figure 4. During the transportation process, the particle is exposed to antimicrobial enzymes in mucus.

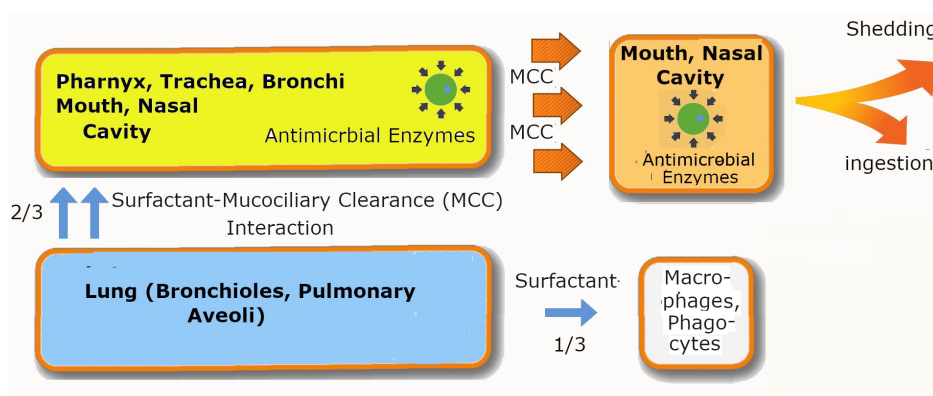


Figure 4. The Mucociliary Escalator/Clearance (MCC) System. MCC consists of a network of mucus-covered structures found in ciliary cells. These mucus-covered structures would push foreign materials away from the lungs towards the mouth and nasal cavity. MCC is absent in the lungs, where mucus is replaced by surfactants. About 2/3 of foreign matters in the lungs are expelled into MCC. The rest are engulfed by macrophages or remain in the lungs.

The three main types of cells in the respiratory tract are ciliate cells, goblet cells, and basal cells. We know that the ciliate cells, which are mucus-coated cells with hair-like structures that are used to move particles away from the lungs, while goblet cells produce the mucus [80]. In contrast, however, the lungs have different types of cells that don't contain mucus. It is likely that the antimicrobial enzymes in mucus and saliva are too harsh for the delicate function of the lungs. Instead, the mucus is replaced by surfactant. The cells found in the lungs are alveolar type I cells (AT1), alveolar type II (pneumocytes, AT2), and macrophages. AT1 secretes surfactants [91-92]. While surfactants are known to have some antimicrobial properties, they are incomparable to the myriad of antimicrobial enzymes found in the mucus.

Given these physiological mechanisms, we can now understand how the host body modulates in the spread and virulence of SARS-CoV-1/2 as seen in Figure 3. When SARS-CoV-1 infects a patient, the greater N disorder allows for the rapid replication of greater quantity of viral particles throughout the respiratory system. The particles are, however, subjected to MCC, in which the particles are swept upwards towards the nasal cavity, but by the time it reaches the nostril, much of the infectious particles are eliminated or damaged due to the comparatively less rigid outer shell, M. The particles that are produced in the lungs tell a different story. Experiments have shown that the lungs do partially participate in the MCC process by pushing 2/3 of foreign particles into the respiratory tract [93]. Since 1/3 of the particles originally in the lungs stay in the lungs, the remaining particles could pose a danger to the patient by damaging the lungs, especially when there are many of them. SARS-CoV-2, on the other hand, produces a more moderate amounts of particles throughout the respiratory system, and many of the particles that are transported to the nostril via MCC remain undamaged, unlike in the cases of SARS-CoV-1, since the former has an abnormally hard M.

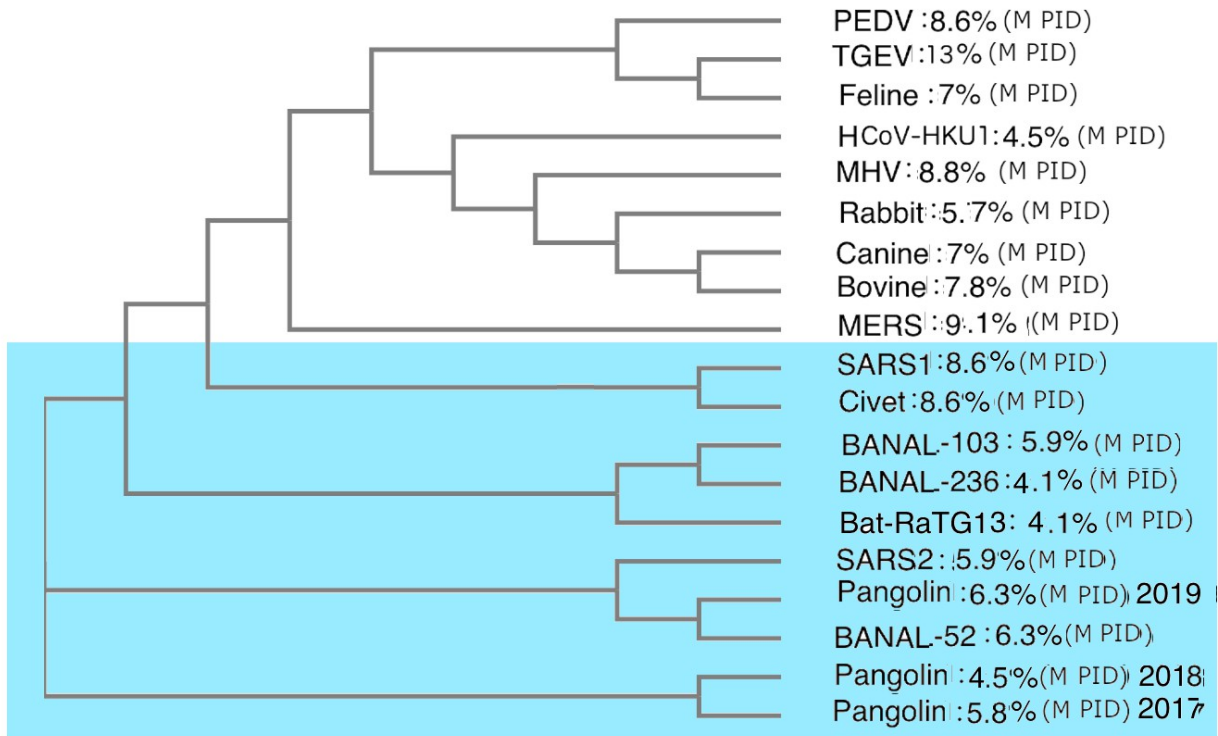
3.7. Phylogenetic Trees Using M Reveal a More Intimate Relationship Between Pangolin-CoVs and SARS-CoV-2: Another Sign of a Pangolin Footprint

We have seen that a hard M is the hallmark of all thus-known COVID-19 viruses. Being hard or ordered usually entails a more conserved protein. Based on this, M is very likely to be highly conserved among all COVID-19 related viruses. Such a feature makes M more ideal for phylogenetic studies, as it is known that recombinations could cause gross errors in phylogenetic calculations. Interestingly, phylogenetic calculations yielded results that are different from those using the entire genome or other proteins [41-48,52,55]. Figure 5A,C uses M to show that pangolin-CoVs have a much closer relationship to SARS-CoV-2, not seen when other protein such N (Figure 5B) or entire genome is used.

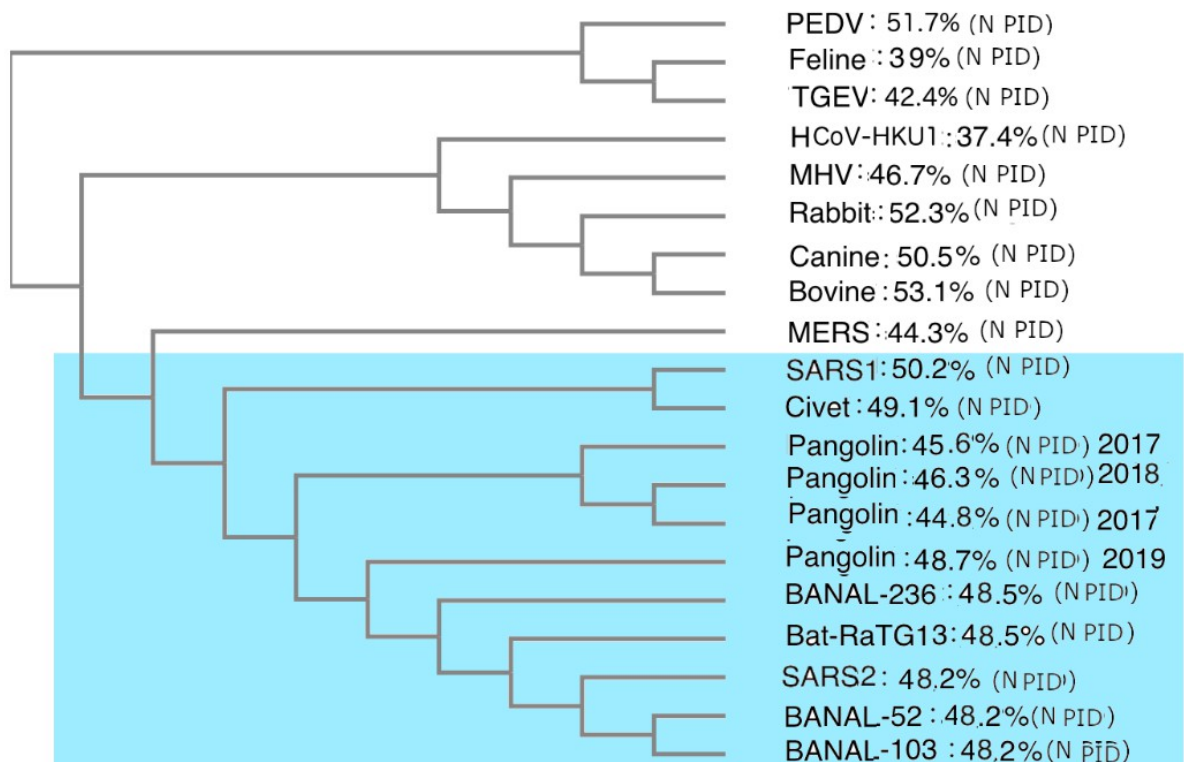
Figure 5A is different from Figure 5C as they involve slightly different algorithms. In any case, both show that pangolin-CoVs have intimate relationships with SARS-CoV-2. It is interesting to note that the two viruses that have the greatest sequence similarities to SARS-CoV-2 are bat-CoVs, RaTG13 and BANAL-52, with 96.1% and 96.8% respectively [51-52]. What is intriguing, however, is that in Figure 3A, it can be seen that BANAL-52 and Pang2019 have the similarly closest relationship to SARS-CoV-2, in contrast to RaTG13. How can this be when RaTG13 has a 96.1% sequence identity to SARS-CoV-2, compared to about 90% for Pang2019? Sequence identity does not offer the full picture because of the possibility of recombination occurring. Furthermore, phylogenetic genetic algorithms tend to make mistakes when recombination had taken place [94]. The abnormally hard M found in all COVID-19-related viruses entails a structural and genetic conservation, which means that the likelihood of any recombination having taken place is much lower. Therefore, we believe that Figure 5A,C presents the most accurate phylogenetic study by avoiding the chances of recombination.

Yet another odd feature can be seen in Figure 5C, where Omicron is more closely related to the pangolin-CoVs than to the other variants [40,52,55]. While this may seem odd, we know that Omicron itself is shrouded in mystery. When Omicron was first sequenced, it was found to have mutations that are unlike any other variants. The question that quickly arises is: Where has Omicron been hiding all along? [95]. Why didn't the medical and scientific community notice it if it was in the human population? There were a few suggestions. A few scientists suggested that the virus was hiding in a small group of immunocompromised people such HIV or cancer patients [96]. Others have suggested that the virus had been hiding in an animal such as rat or pangolin. One paper has suggested that Omicron had been hiding among mice based on the mutations of its S and mouse ACE-2 [96]. Disorder studies on Omicron do suggest that it could be hiding in a burrowing animal such as pangolin as the first Omicron variant BA1 had an even harder M than other SARS-CoV-2 variants (M PIDs: 5.4% Vs 5.8%) [55]. The idea that Omicron had been hiding in mice actually does not contradict the suggestion in the previous statement since mice are also burrowing animals. A complication arises, however, when we examine the evolution of mice and rats. While rats and mice dwell in burrows in the countryside, rats and mice in the urban settings have evolved to live in the homes of human (Schmidt-Holmes et al) [97]. Therefore, depending on the species, they could have characteristics of both burrowing and non-burrowing animals. The phylogenetic tree (Figure 5C) that shows an unusually close relationship between Omicron and pangolin-CoVs, in contrast to the other variants, adds an important clue towards solving the puzzle. The phylogenetic tree is suggesting that it was literally hiding within a population of a burrowing animal such as pangolins.

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COVID-19 will be uncovered as well [102-103]. Contrary to the popular notion, there is mounting clinical and experimental evidence that the S protein may not be the main underlying cause of COVID-19 infectiousness and virulence. One major piece of evidence for this lies in a comprehensive clinical study conducted by Wolfel et al [29]. In this study, it was found that COVID-19 patients shed much more infectious particles than SARS-CoV-1 patients. If we look closely, the question that comes to mind is: why does SARS-CoV-2 need much more particles to be more infectious if its S has a 10 or 1000 times [9-11] greater binding affinity to ACE-2 than the latter? Keeping in mind that it requires much more energy to produce these extra infectious particles and nature does not, as a rule, usually waste energy to do redundant things. One could, however, attempt to get around this paradox by claiming that the S affinity to ACE-2 is responsible for the more rapid replication of the virus. Unfortunately, this suggestion contradicts what we know about the life-cycle of the virus, as viral entry represents only an initial stage of a long series of process that includes RNA replication, protein production, assembly, packaging, and the budding of viral particles [106-109].

There are those who suggest that it is possible that SARS-CoV-1 S binds more efficiently to the ACE-2 in cells in the lower respiratory in contrast to SARS-CoV-2 [12]. There are a number of issues that arise when such an argument is made. Firstly, the argument is made without any considering current physiological knowledge, namely the Mucociliary Clearance system (MCC), which allows viral particles produced throughout the respiratory system to be transported upwards towards the nasal area so that they could be expelled and shed [88-91]. Secondly, to our knowledge, attempts to reproduce the argument involving SARS-CoV-1 S and ACE-2 has not been conducted experimentally, probably, because of the current difficulty in obtaining the now extinct SARS-CoV-1 to conduct comparative experiment alongside SARS-CoV-2. There are, however, also similar arguments made to account for the differences in virulence of Omicron and non-Omicron variants Hui et al [78]. This difference is that COVID-19 pandemic provides us with a deluge of clinical and experimental data. In fact, attempts to reproduce this argument has been made, which we will discuss at length later.

The clinical study of Wolfel et. al. [29] is just the tip of the iceberg, as there is also a deluge of other evidence that shows that the nature of S is not what many scientists have made it out to be. Nor does S alone provide for a coherent conceptual framework of COVID-19 infectiousness and virulence, unlike M and N. This is also the case with long COVID. We will examine these in greater details in later sections. This is not to say that S is unimportant or that it does not play any part in infectiousness or virulence. It is important, but we must also fully comprehend its true potential and limitation. A more complete understanding of the true limitations and potentials of S will come when we study other important proteins such as N and M more thoroughly, which is challenging even if the majority of research is oriented towards S.

4.2. SDMs and Reproducibility

We have seen that SDMs are highly reproducible by their ability to accurately predict and explain certain phenomena that are otherwise difficult to account for. Unlike alternative explanations, they are explained in a coherent manner using a logical and unified paradigm that is consistent with current knowledge of physiology and biochemistry [52]. There is also important clinical and experimental evidence that reproduces many of the predictions of SDMs.

While a previous experiment was not able to detect any statistical difference in the ability of SARS-CoV-2 to last on various surfaces under presence of light when compared to SARS-CoV-1, Riddell et al. [21] conducted a similar experiment, devoid of light, and found that SARS-CoV-2 lasts much longer on external surfaces than the control CoVs. SDMs predict that SARS-CoV-2 is more persistent than most virus as its unusually hard M protects against the environment and harsh antimicrobial enzymes, and this experiment reaffirms SARS-CoV-2's resilience in the absence of light.

Reproducibility of SDMs is not confined to computational and experimental research, but also extends to clinical studies. One such study involved an investigation into the infectiousness of SARS-CoV-19. It was found

that COVID-19 patients shed a much larger amount of infectious particles than the 2003-SARS patients [29]. This contradicts all other paradigms set forth. For instance, if S is fully responsible and has a much greater affinity for human ACE-2, why does SARS-CoV-2 need to expunge such higher quantities of particles in order to be more infectious? SDMs provide for a much more elegant explanation: the virus is more resistant to the salivary and mucosal anti-microbial enzymes because of the abnormally hard SARS-CoV-2 outer shell (low M PID). This clinical observation raises more questions than it answers. More specifically, how is the virus able to make the host expunge such a large amount of particles without being more virulent to the body? If we assume that the body is shedding more viral particles because it is producing more virus copies, then vital organs such as the lungs should be flooded with the virus, thus making the latter more dangerous, but this is apparently not happening in the case of COVID-19.

Adding to the paradox, Ogando et al. [79] found that SARS-CoV-1 has a higher viral growth VERO-E6 cells than SARS-CoV-2 under the same conditions. Even if this is consistent with the greater pathogenesis of SARS-CoV-2, how do we then reconcile this result with the previously mentioned clinical observation? If SARS-CoV-2 S has a 10 or 1000 times [9-11] greater affinity for ACE-2 than the affinity between SARS-CoV-1 S and ACE-2, how is this possible? It seems that we need to look elsewhere for answers by examining N and M more closely. The SDMs explain that the reason that SARS-CoV-1 is producing higher levels of particles has to do with the higher N disorder that allows greater efficiencies in its replication process but, conversely, lesser infectious particles are shed by the body as the higher M PID does not provide sufficient protection against the antimicrobial enzymes.

Yet another enigma involves long COVID [110-111], and SDMs are able to explain the persistence of the virus among COVID-19 patients even months after infection. Again, we return to the theme of hard M that protects the virus from antimicrobial enzymes. This time the focus is not only on mucosal and salivary enzymes as in the case of infectivity, but also on antimicrobial enzymes in the immune system. Further discussion on the antimicrobial enzymes found in the immune system can be found in the long COVID section below.

4.3 More Reproducibility: Omicrons and Pangolin-CoVs

There are many other predictions that SDMs make involving N and M. One such prediction pertains to pangolin-CoV. When SDMs were applied to pangolin-CoVs, it became obvious that the 2017 pangolin-CoV (Pang2017) isolate from Guangxi is attenuated because of its low N PID (~44%) [40,52,55-56]. There are several implications for this finding. If this or a similar virus had entered the human population, it is likely that it would have easily moved quietly among humans as a mild cold without the notice of the medical communities [18]. The predicted attenuation has been independently reproduced by several laboratories [52,55,74-77]. Animals models have seen milder manifestation of symptoms upon Pang2017 infection, in contrast of the Wuhan-Hu-1 strain. The SDMs prediction pertaining to Pang2017 was published before the arrival of Omicron.

Omicron was first detected in South Africa around November 2021. Omicron was clinically and, later, experimentally observed to be milder than previous variants [71-78]. Once again, SDMs have much to say. Based on the initial Omicron subvariant, BA.1, the N and M PIDs are 43.65% and 5.4%, which are both lower than previous variants [49]. The smaller than usual M PID (5.9% vs. 5.4%) could suggest that the virus had a recent origin involving a burrowing animal, whereas the lower N PID predicts that Omicron is likely attenuated to similar levels as Pang2017. The prediction involving N PIDs was experimentally replicated when it was shown that the viral growth of VERO-E6 cells infected by Pang2017 and Omicron respectively are very similar, and when it was shown that the viral damage on cells by the two viruses are similar [55]. This presents evidence of further reproducibilities for both Pang2017 and Omicron. Omicron has also been shown to be attenuated with lower growth than Wuhan-Hu-1 under viral titration [55,75,77,79].

The correlations between N disorder and viral titer or virulence can be found in SARS-CoV-2 related virus data that include Pang2019 [71-73,75] and the Laotian bats-CoV (BANAL) [50-51]. Furthermore, while Pang2017 has been predicted and reaffirmed to be attenuated, this is not the case with Pang2019. Instead, SDMs have to predict Pang2019 to be non-attenuated with its N PID at 48.2% [18,52,55-56]. Huo et al. [81] were able to obtain levels of viral titer from Pang2019 similar to those of Wuhan-Hu-1 in VERO-E6 cells. Several laboratories were also able to independently observe that Pang2019 is able to inflict severe disease in at least one strain of mice. In contrast, virulence was not observed in Pang2017 [55,74-79]. All these are again consistent with the predictions of SDMs. We know that all of these SARS-CoV-2 related viruses do not possess FCS, unlike SARS-CoV-2. The question then becomes: how does Pang2019 possess similar infectivity and virulence as SARS-CoV-2 without FCS, when FCS-mutant non-Omicron SARS-CoV-2 is largely not transmissible among ferrets?

4.7. Measuring Virulence Using CFR, Animal Models and Cell/Tissue Damage Observation

While Figure 1 shows correlation between Inner Shell Disorder and CFR, it must be admitted that CFR is not necessarily the most ideal representation of virulence for at least two reasons. Firstly, CFR figures are often extrapolated out of necessity [103]. Secondly, CFR is applied only to human, not animals. Furthermore, virulence may vary among different animals even within a single virus or variant. There are, however, other methods of measuring virulence such as animal models, viral titration and indications of cell/tissue damage after infection. We have mentioned some of the animal models. It must be noted that attenuation or aggressiveness of SARS-CoV-2 variants, Pang2017-CoV and Pang2019-CoV were reproduced by viral titrations, animal models and inspections of cell/tissue damage after infecting cells or animals were conducted by at least two independent laboratories for each virus or variant [55,74-79]. The animal models included, at least, hamsters and several strains of mice. In addition, the severity of Pang2019-CoV infection was also observed in pangolins under laboratory conditions [80-82]. It can also be argued that a SARS-CoV-2 related virus may infect different types of cells in different manner, which could potentially make interpretation of viral titration data more challenging. This is related to the argument made by some scientists that SARS-CoV-1 could infect the lower respiratory tract more efficiently in comparison to SARS-CoV-2. There are, however, hints that the arguments may not present a great difficulty in the interpretation of viral titration data. Viral titrations have been made a variety of cells including Vero E6, Calu3 and Caco2 [77]. The data show subtle but definite differences in the viral titers using the three different cell lines that is the result of differences in S-ACE-2 binding mechanism. The differences, however, are not so huge that it would make interpretation of viral titration data challenging as the variations viral titers among cell types are not great and follows a trend. Besides, as we see later, the S of SARS-CoV-2 related viruses typically become quickly more efficient to binding to the S of different cell types after several passages. With all these in mind, it is possible to present a consistent picture of virulence nad infectivity. In fact, a previous paper [55] was able to find a positive correlation between virulence and N PIDs based on a combination of data from viral titer, animal model and cell plaques.

5. Comparative Roles of S, M, and N Proteins

5.1. The S Protein, Omicron, and Pangolins

We have seen that the use of S is unable to explain some crucial experimental and clinical data. This just the tip of the iceberg, as there is more experimental and clinical data that S alone simply cannot explain or account for and, thus far, attempts to do so are not reproducible, or can be shown to be inherently flawed as we have argued. There are also important biological reasons for this. For us to understand this, we need to examine the basic fundamental biology of the virus. While S is definitely an important protein in terms of viral entry, there is no fundamental reason that S should be the most important protein in COVID-19 infectiousness and virulence, as much as many scientists would like to believe. Viral entry, though important, is just the first of the many steps in the virus' life-cycle [109]. It must be emphasized that

SARS-CoV-2 encodes 19 proteins, even though not all are major proteins [109]. S is not the only major protein, and neither is it the most abundant protein. On the contrary, the N and M are the most abundant major structural proteins found in the cell and virion respectively [96-100]. For this reason alone, we should not be surprised if N and M have greater influence on the infectivity and pathogenesis of the virus.

Further sets of perplexing evidence that contradict the “S-alone” paradigm can be found in the data that pertains to pangolin-CoVs and Omicron. We need to keep in mind that all variants of SARS-CoV-2 have FCS that is absent in all COVID-19-related bat and pangolin viruses. Many scientists thought that they have found FCS to be the true cause of COVID-19 infectiousness and virulence when it was found that SARS-CoV-2 with FCS intact is more aggressive in respiratory cells, in contrast to the FCS-mutated one, by promoting cell to cell fusion. Furthermore, it has been shown that while non-Omicron SARS-CoV-2 wild-type can be transmitted experimentally via aerosol between hamsters, no transmission was seen when FCS-mutant was used on ferrets [113]. Again, how can this be the case, given that pangolin-CoV has no FCS and is easily transmitted between hamsters [74-75,81] ?

5.2. The S and Omicron Conundrum

When Omicron arrived, it presented an enigma by being infectious and yet attenuated. How did Omicron achieve its attenuation given that it has FCS just like all other variants? Animal studies have shown that rats are unable to transmit via aerosol in the laboratory. How could this be so when Omicron has been clinically shown to be highly infectious? More importantly, how can this be happen when Omicron has efficient FCS [114-115], in which its presence in other variants has been shown to induce greater transmissibility in ferrets [113]? These discrepancies point to the probability that other factors are in play. In fact, all these, as we have shown, are consistent with the workings of N and M, as summarized in Figure 5.

An attempt to get around this “S-Omicron” paradox is the hypothesis that claims Omicron infects the cells in the upper respiratory system more easily than the lungs. Hui et al. [78] were able to isolate bronchial and lung tissues, which were infected with Omicron and previous COVID-19 variants. They were able to qualitatively see the greater presence of viral particles and concluded that Omicron is attenuated because it replicates more easily in the lungs than in the bronchi. There are, however, a number of problems with this interpretation. Firstly, this observation has not, to our knowledge, been reproduced in other laboratories. In fact, several laboratories have observed efficient replications in both lungs and upper respiratory systems [77,116-117]. Furthermore, several other independent laboratories have noticed that many subvariants of Omicron are even more adapted to the human ACE-2 than the non-Omicron variants [116-118]. If this is the case, why hasn't Omicron achieved greater virulence and infectivity like its predecessors?

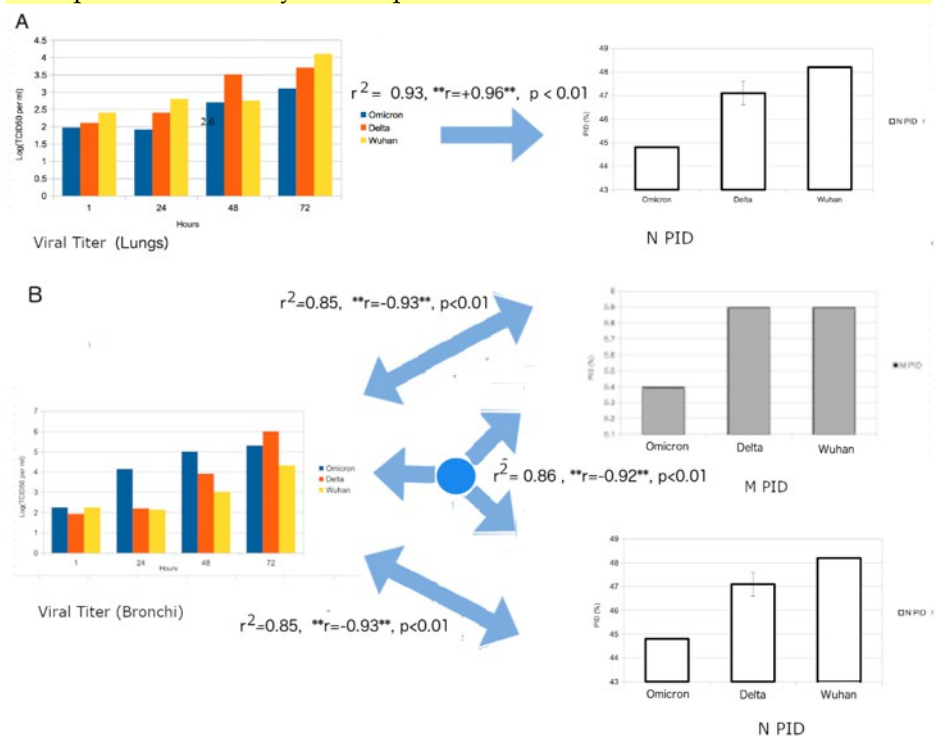
5.3. Evidence of the Different Roles of N and M in Experimental Data

The story is actually even more complicated than what Hui et al. had envisaged [78]. In reality, the N and M proteins, together with knowledge of MCC, provide a more consistent and reproducible explanation for their results. In our previous publications, we have shown that the initial waves of Omicron ha lower M PIDs than previous variants. This feature is likely a tell-tale sign of its recent interactions with a burrowing animal, possibly, the pangolin. In any case, it explains why Hui et al. were able to see more viral particles in the bronchi. It is because Omicron is more resistant to the anti-microbial mucosal enzymes that it encounters as it is being transported upwards by the hair-like structures in the network of mucus-covered ciliary cells. The lung has no ciliary cells or mucus but has surfactants that, even though it is anti-microbial, is not as harsh, unlike the variety of enzymes present. The apparent lack of viral particles is likely an indication of particles still trapped in the tissues as there would not be MCC to bring it to the top in the lungs [88-92] .

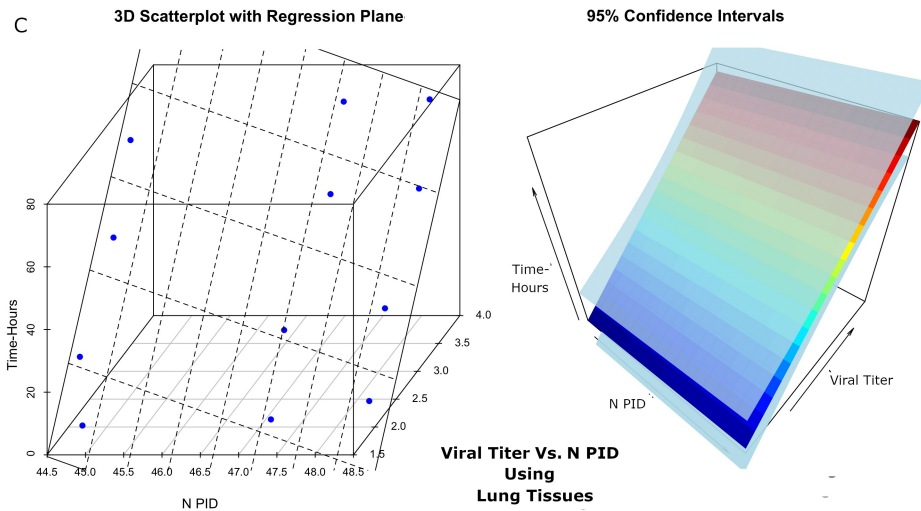
Viral titrations were also made using Omicron, Delta, and Wuhan-Hu-1 in lungs and bronchial tissues in the above-mentioned experiment. The data were used to support the hypothesis of differentiated replication of Omicron in the

lungs and bronchi. In one of our previous articles, we managed to use their data to perform multivariate analysis (Figure 6). The viral titration data were taken from publicly available paper of Hui et al, while N and M PIDs based on variants and sub-variants were taken from our curated disorder database (Table 2). Regression (Multivariate) Analysis was performed using R package. The regression analysis provides us with the correlations between viral titer and N/M PIDs is measured as correlation coefficients (r) or coefficients of determination (r^2). The total n (sample size) for the entire statistical experiment as seen in Figure 6 is 24 ($p < 0.01$, $n = 24$, $r^2 \sim 0.9$). Statistical results were computed using R package that is available publicly [119-120].

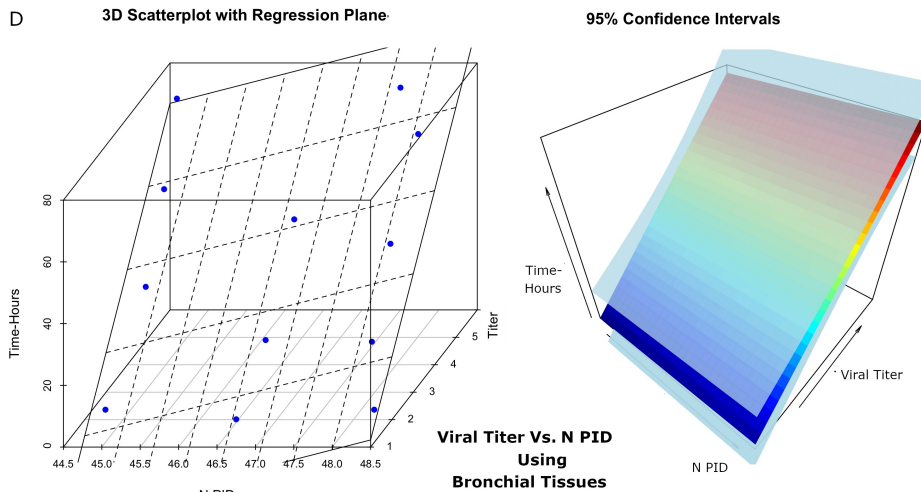
We found strong correlations between N/M PIDs and viral titers. We were also able to observe peculiar changes in the signs of the correlations when moved from lung tissues to bronchial ones [40]. We were able to obtain a positive correlation ($r = +0.96$, Figure 6A) especially on the N PID and viral titer ($VT = A * PID_N + B * Time + C$, where A,B,C = coefficients and VT = viral titer). It was, however, very startling when we received a negative correlation ($r = -0.93$, Figure 6B) between PID_M/PID_N and viral titer ($VT = A * PID_M + B * PID_N + C * Time + D$ where VT = viral titer, A,B,C = coefficients and D = Y-intercept) [44]. What is remarkable and puzzling is the change between the positive and negative signs. It is not only reproducing the SDMs but also instructing us on how to use the SDMs. The change in the sign is an indication that the greater presence of particles in the lungs is dependent on the greater N disorder (higher PID_N), whereas the greater viral presence in the bronchi is dependent on lower disorder in N and M PID (lower M PID and N PID). The change in the direction of the slopes (correlations) can be observed in Figure, 6C-D. We will also see that this change in slope (correlation) is completely absent when viral titration is conducted in an animal model, instead of tissues (Figure. 7), as virus particles can easily travel upward via MCC in the case of animal models.



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Figure 6. Multivariate Analysis of M/N Disorder and Viral Titer. **A.** Regression analysis reveals a positive correlation between N PID and the viral titer from human lung tissues and N PID. (Model: $VT = A*(N\ PID) + B*Time + C$ where $VT =$ Viral Titer, $A,B =$ Coefficients, $C =$ Y-Intercept.). **B.** Regression analysis found a negative correlation between viral Titer from human bronchial tissues and M/N PIDs. (Regression model: $VT = A*(M\ PID) + B\ (NPID) + C*Time + D$ where $VT =$ Viral Titer, $A,B,C =$ Coefficients, $D =$ Y-Intercept). **C.** The three dimensional plane with the 95% confidence interval as applied to the regression analysis involving viral titration on the lung tissues. **D.** Three dimensional plane with 95% confidence interval related to the the viral titration using bronchial tissues. As (C) and (D) provide only three dimensional representations (Model: $VT = A*(N\ PID) + B*Time + C$ where $VT =$ Viral Titer, $A,B =$ Coefficients, $C =$ Y-Intercept.), they do not offer a complete picture that can only be found in four dimensions(Regression model: $VT = A*(M\ PID) + B\ (NPID) + C*Time + D$ where $VT =$ Viral Titer, $A,B,C =$ Coefficients, $D =$ Y-Intercept). The analysis is a statistical extension of the experiment of Hui et al. [40,43]. Data pertaining to viral titration and PIDs are from the the experiment of Hui et al and available in Table 2 (which can also be found in previous publications) respectively. A negative slope (correlation) with respect to the N PID-Titer axes can be found in (D), unlike (C).

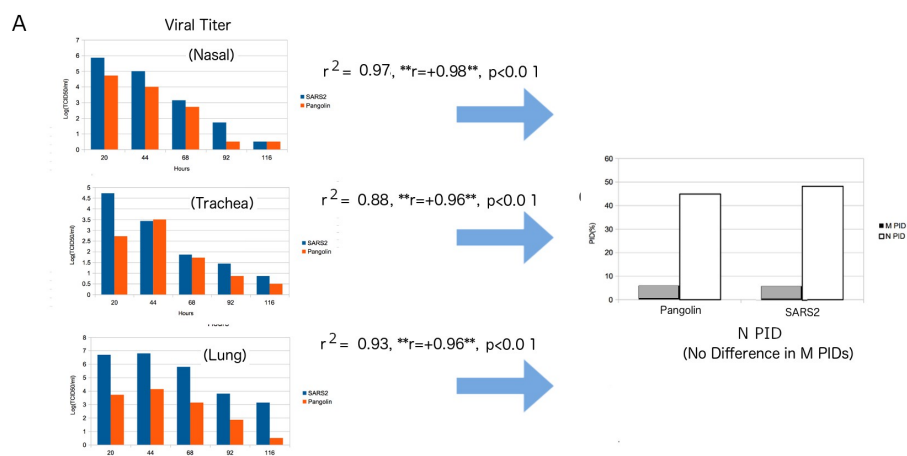
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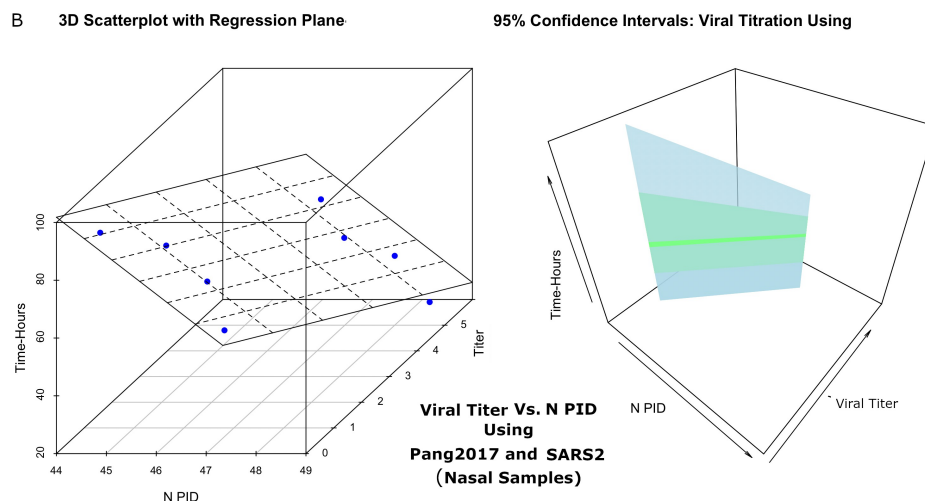
Prior to this investigation, we focused on the hard outer shell (low M PID) in resisting the onslaught of mucosal antimicrobial enzymes and on greater N disorder in its ability to assist in providing more efficient viral replication [18-20], but the experiment also showed us that N plays a role in protecting the virion from damage [52,55]. This is something that we had forgotten about, even though we had evidence from the very beginning that harder inner shells do protect the virus from harm in viruses such as EIAV, DENV, and rabies [34-35,55-56,65-67].

We conducted a similar regression study as seen in Figure 7 using the experimental data of Guo et al. [75]. Again, we were able to obtain the titration data from the published paper of Guo et al with the respective N and M PIDs,, which depends on the SARS-CoV-2 variant and pangolin-CoV isolate, obtained from our curated database. As with the data from Hui et al, we did a regression analysis to obtain the correlation coefficients (r) and coefficients of determination (r^2) grouped by the location in the respiratory system the samples were obtained (Figure 7, Total n = 30, $p < 0.01$, $r^2 \sim 0.9$).

Guo et al. used Pang2017 and hamsters in lieu of Omicron and tissue cultures respectively [65]. Therefore, M becomes an unreliable independent variable as there is hardly any difference between the M PID of Pang2017 and non-Omicron SARS-CoV-2. We also expected to see the full effect of MCC since Guo et al. [75] used an animal model, in contrast to the use of tissues by in Hui et al. [78] i.e. the virus particles are more able to move freely between different parts of the respiratory system via MCC. Positive correlations between N PID and viral titer were seen in samples collected from all three areas of the respiratory system as seen in Figure 7, which helps validate SDMs and the results described in the previous sections. Figure 7B affirms a positive slope (correlation) when view with respect to the N PID-Titer plane.



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Figure 7. A) Multivariate analysis of N/M disorder (PID) and viral titer of pangolin-CoV/SARS-CoV-2 in VERO-E6.(Regression: $VT = A * (N \text{ PID}) + B * \text{Time} + C$ where $VT =$ Viral Titer, $A, B =$ Coefficients , $C =$ Intercept). **B.** A three dimensional regression plane and confidence interval with viral titer data from the nasal samples. The results is a statistical extension of the experiment of Guo et al [40,75]. Viral titration data used are from the experiment of Guo et al, whereas PIDs data are available in Table 2 and previous publications. A positive slope (correlation) with respect to the N PID-Titer plane can be seen in (B).

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5.4. Hui et al Experiment: S-Alone Hypothesis Vs. SDMs

The S-alone hypothesis argues that SARS-CoV-1 infects the lower respiratory tract more easily as its S binds more efficiently to the cells in the lower respiratory system. The same argument has been made to account for the lower virulence of Omicron compared to other variant. Hui et al tried to show exactly this. They grew the various variants in bronchi and lung tissues. Their viral titration data show lower viral titers for Omicron in the lungs than bronchial tissues. When we used their viral titration data to do more elaborate statistical analysis to correlate the viral titers to N and M PIDs, our results tell us, however, something different [40]. There are strong correlations between viral titers and N/M PIDs in both bronchial and lung tissues. Not only that, positive and negative correlations were found in the lung and tissues respectively. The results not only reproduce the predictions of SDMs but taught us how to interpret SDMs. The positive correlation between N PID and viral titer in the lungs tells us the greater presence of viral particles is due to the great N disorder (PID), which depends on the variant keeping in mind that the lungs have surfactants, in lieu of mucus. Surfactant has some antimicrobial effects but not the strong array of antimicrobial enzymes present in mucus. That is the reason that there is a strong positive correlation of viral titers in the lung tissues with N PID and small correlation with M PID. The puzzling thing is then: Why is there a negative correlation between viral titer in bronchial tissues and N/M PIDs? The answer has to do with the fact, in contrast to the lung, bronchial tissues, like most upper respiratory tissues, have mucus. A

negatively correlated M PID means that the hard M is protecting virus from anti-microbial damage. What we did not expect is a correlation with N disorder. It was then we realized this result is telling us that N also plays a role in protecting the virion from damage especially when exposed to an onslaught of antimicrobial enzymes. The results is actually teaching us how to interpret predictions using SDMs. Indeed, we had previously observed that some viruses, such as the rabies virus (RABV), with harder inner and outer shells are more thrive better in harsh environments such as exposure to saliva [65-67]. It must also be noted that M and N bind to each other in close proximity in the virion. Further literature search has provided evidence experimental evidence that M and N together allows greater structural integrity by the non-covalent binding between the two proteins. This renders support to the computational results that suggest both M and N protect the virion from damage.

What our more elaborate statistical analysis is telling us is that the higher viral titer of Omicron is not due to the difference in the greater presence of viral particles in the bronchial tissues is not due to the differences in S but due to the greater ability of the virus in resisting damage from the greater presence of mucosal antimicrobial enzymes in the bronchial tissues. Omicron's greater resistance to anti-microbial enzymes arises from its greater hardness in both M and N (N PID: 44.8%, M PID: 5.4%). The particular Omicron variant used was BA1, which was the original subvariant that was first detected in South Africa. While all Omicron variants have lower N PIDs, BA1 has the peculiar characteristic of even lower M PID (~5.4%) than all other variants or other Omicron subvariants (~5.9%) [55]. As a result of this and its harder N, it is able to resist the antimicrobial enzymes in the bronchial tissues and thus there is a greater presence of viral particles. In the lung tissues, however, there is a lesser presence of antimicrobial enzymes but because Omicron N PID is much lower it is unable to replicate as fast as the other variants especially in the lungs. This is what the more elaborate statistics is detecting, and this result is arguably more plausible than the one presented by Hui et al [78] as the former shows a more intricate reason for the results of the latter.

5.5. The Role of MCC in Virulence and Infectivity

Puzzling and seemingly contradictory results is seen in the viral titration of pangolins as seen in Figure 7 (Guo et al [75]). Unlike the statistical findings from Hui et al data, the statistical analysis using the data from Guo et al [75] shows positive correlations for viral titers to N PIDs in both upper and lower respiratory tracts. This may seem like it is contradictory to the results we have just discussed, but it is really not. The experiment conducted by Guo et al was based on live hamster infected with pangolin-CoV. They measured the viral titers by extracting samples from euthanized hamsters. Hui et al, on the other hand, relied solely on viruses grown in bronchial and lung tissues respectively. Therefore, we should expect the full effects of MCC in experiment of Guo et al, but only limited MCC effect in the experiment of Hui et al. Because the viral particles produced all over the respiratory system including the lower respiratory system will move upward towards the nasal region for expulsion. That is exactly what the statistical result in Figure 7, unlike Figure 6, is telling us. The results of Figures 6-7 do cast doubt on the hypothesis that SARS-CoV-1 is less infectious but more virulent is caused by the ability its S to bind more easily to the ACE-2 cells in the lower respiratory tracts because MCC entails that much of the particles produced even in lower respiratory tracts to be transported upwards to the nasal region to be expelled from the body. The same argument has been made for the more virulent SARS-CoV-2 variants. It seems that a more plausible scenario is that the virus replicates about the same amount throughout the respiratory system. The more virulence strain or variant with higher N PID replicates in larger quantities throughout the respiratory system especially in the lungs but whether much of the particles is expelled from the body depends if the virus is resistant to the onslaught of mucosal and salivary antimicrobial enzymes via a harder M.

5.6. SDMs Account for MCC: Virulence Vs. Infectivity

One problem with the "S-Alone" hypothesis involving SARS-CoV-1/2 virulence and infectivity is that it is oblivious of current physiological knowledge, namely MCC. Even if SARS-CoV-1 does bind more efficiently to the lower respiratory tract compared to upper respiratory tract and thereby making SARS-CoV-1 less infectious

but more virulent, MCC entails that the viral particles will move upward towards the nasal region to be expunged. This is, of course, assuming that the viral particles will survive the onslaught of anti-microbial enzymes during the transportation by mucus-covered ciliary cells. The MCC factor has to be taken into account if we attempt to demonstrate the difference in virulence and infectivity between Omicron and non-Omicron variants. Because MCC is an important principle in physiology, MCC must therefore be essential in explaining the mechanisms for virulence and infectivity manifestations. SDMs, unlike the S-alone hypothesis, able to account for MCC and MCC can help explain the link between infectivity and virulence via SDMs. MCC and SDMs therefore compliment each other to account for the differences in infectivity and virulence manifestations of SARS-CoV-1/2 and SARS-CoV-2 variants.

5.7. *The HCoV-NL63 Enigma*

The above sections do not show that S does not play any role in infectivity and virulence but, rather, that S does not play the roles in infectivity and virulence in many of the ways we think it does. Its roles should be seen in the broader context alongside other proteins such as N and M. To understand it further we turn our attention to another human CoV. The only other known human CoV (HCoV), other than SARS-CoV-1/2, that binds to ACE-2 is NL63 [121-126]. HCoV-NL30 usually manifests itself as a mild cold even though it known to be capable of infecting both the lower and upper respiratory tracts. NL63 and SARS-CoV-1/2 are not closely related as they are alphacoronavirus and betacoronavirus respectively [126]. It can be argued that NL63 is somewhat less infectious than SARS-CoV-2 since the former usually infects only children and immunocompromised individuals such as the elderly, unlike the latter.

NL63 has no FCS-recognition site even if a FCS sequence has been detected at S2 [123-124]. While cleavage is seen at the Golgi apparatus of cells infected by SARS-CoV-2, it is not seen in NL63 infection. Keeping in mind that SARS-CoV-1 does not have FCS, unlike SARS-CoV-2 [101-103], the absence of an FCS recognition site presents an intriguing enigma. If NL63 and SARS-CoV1 have no FCS-recognition site, why is NL63 obviously less infectious than SARS-CoV-1? Conversely, if SARS-CoV-2 has an FCS-recognition site, unlike NL63 and SARS-CoV-1, why does SARS-CoV-2 has an infectivity that is comparable to NL63, when compared to SARS-CoV-1, given the fact that both COVID-19 and NL63, not SARS-CoV-1, are endemic? We know that SARS-CoV-1 has limited infectivity as there was only 8,422 known cases of infection and it is now extinct, whereas COVID-19 and NL63 is endemic even to this day [3,12126]. All these seem to contradict the idea that the presence of FCS is responsible for higher infectivity [102-103]. As for pathogenesis, why are SARS-CoV-2 and NL63 much less virulent than SARS-CoV-1 when SARS-CoV-2 is the only virus of the three that has FCS-recognition site, given that the presence of FCS is associated with greater pathogenesis?

Likewise, the relationship between S-ACE-2 affinity and pathogenesis or infectivity can cast much confusion especially when NL63 is taken in to consideration. As we have seen the affinity of SARS-CoV-2 S to human ACE-2 is much higher than that of SARS-CoV-1. Many scientists have postulated that this discrepancy is responsible for SARS-CoV-2 higher infectivity especially among humans [9-11]. This postulation is, however, unreplicable when it has been shown that NL63 S affinity for ACE-2 is less than that of SARS-CoV-1 [126]. How could this be possible when it NL63 is obviously much more infectious than SARS-CoV-1 for the reasons already mentioned? All these questions form a conundrum that is difficult to be explained by S alone and is more effectively explained when the different roles of S, N and M are taken into consideration.

This virus is, however, also enigmatic for SDMs as its N disorder is very close to that of SARS-CoV-1 (N PIDs: 49.8% vs 50.2%) but NL63 is, of course, not as virulent. Does that mean that SDMs are wrong? If we look closely, we will find that this is not necessarily true. SDMs usually work better when the viruses that are compared are closely related. NL63 is not closely related to SARS-CoV-1/2 as a result the proteins in general are not similar between the two. Because of the differences in proteins especially those other than the shell proteins, other factors can come into play such as differences in toxicities of other proteins. This is a limitation of SDMs.

Nevertheless, in this case, comparison using SDMs is possible in this case. While the NL63 N PID is higher than SARS-CoV-2 but comparable to SARS-CoV-1, the M disorder tells a different story. It is higher much higher than that of SARS-CoV-1/2 (NL63 M PID: ~11%, SARS-CoV-2 M PID: 5.8-54.%, SARS-CoV-1 M PID: ~9%). If you apply what is already known about SDMs, the relatively soft NL63 M and N means that even though relatively much higher viral particles are produced even in the lower respiratory tract, the particles are easily damaged by the mucosal antimicrobial enzymes the moment they are produced. It is for this reason that NL63 is usually not dangerous. If we scrutinize the clinical data more carefully, however, we see that NL63 is capable of infecting both the upper and lower respiratory tracts [121-122] and that the people it infects in this manner are mainly children and immunocompromised adults such as elderly. These clinical manifestations may be the result of the ability to replicate in larger quantities in all areas of the respiratory system if unchecked by the immune system including the anti-microbial enzymes in the mucus. SDMs also suggest that despite its greater vulnerability to damage by mucosal antimicrobial enzymes, some particles are able to reach the nasal region to be shed because greater quantities are produced as a result of a more disordered N. The fact that children and immunocompromised adults are more easily infected could place further support to the idea that only limited quantities of viral particles are shed. Children often play in close contact with each other especially at preschools.

5.8. *The Protective Roles of Outer and Inner Shells*

We have seen how both the outer (M) and inner (N) shells play roles in protecting SARS-CoV-2 from antimicrobial enzymes in the mucus and saliva. Apparently, this is not the only virus that have shown such properties. There are several papers that show that viruses that are exposed to saliva usually have hard outer shells and often have also hard inner shells. Examples of such viruses are EIAV, rabies virus and Zika virus [4,34-35,66-67]. EIAV is transmitted between horses via a horsefly that sucks blood of an infected horse that is store in the insect's mouthpiece containing its saliva before it sucks the blood of a new host, which is thereby infected. The rabies virus resides near the salivary gland of the host and is therefore exposed to saliva. Similarly, ZIKV is exposed to saliva as it is held in the mouth of a Aedes mosquito upon feeding on the blood of an infected host. These, however, do not necessarily mean that all viruses have inner shell hardness even all viruses exposed to saliva usually have hard outer shells. This is the case in the flavivirus family, in which the viruses are usually insect-borne and have hard outer M but a few such as ZIKV have also harder capsid.

5.9. *The Functions of M and N*

A great way to gain more insights into the structure, nature and functions of viral proteins such as N and M is by inspecting the SARS-CoV-2 using microscopy especially electron microscopy (EM) [127-128]. Inspections of the SARS-CoV-2 using powerful EM have revealed the reasons that M and N are the most abundant proteins in the virion and cell respectively. M spans the entire membrane than enclose the entire virion whereas N encases the RNA genome [127-128]. The structural arrangement itself suggests protective roles of the two proteins. M binds strongly to both S and N. The affinities for S and N allows M and N to play important roles in assembly and budding of viral particles [107]. Because of the strong affinity between M and N, greater flexibility in N provides for more efficient recognition between N and M [16,30-32]. This is one of the reasons that greater N disorder allows faster and effective viral replication.

There is yet another property of M and N that has been observed using EM. This involves the nature of of M and N binding. Powerful EM was able to observe that the C-terminus of M binds to N via an ionic interaction [129] and in the absence of this interaction, the entire virion collapses under harsh conditions such high salt or PH. This important observation renders support to the suggestion that M and N play roles in protecting the virion. While M protects the entire virion, N is likely to protect

specifically the genomic RNA by the fact that N encases the RNA and thus there is strong affinities between the two entities [106-109]. The N affinity to the viral RNA has given N many important roles especially those pertaining to viral replication. This includes assembly and RNA transcription [107-109,129-131]. Because of its important multiple roles in replication and the life-cycle of the virus, we are able to see how N disorder can easily affect virulence and infectivity along with M disorder as greater N disorder promotes easier protein-protein/RNA/lipid/carbohydrate recognition [16,30-33,68].

We have tried to show that SDMs is highly reproducible and can account for many of the properties and manifestation of SARS-CoV-2 related viruses and COVID-19. Such reproducibilities should not be surprising as M and N are the most abundant proteins in the virion and cell respectively and they play major roles in the life cycle of the virus especially with regard to its replication [106-109]. Nevertheless, we need to keep in mind that M and N are, of course, not the only viral proteins in CoVs. While they play major roles in the replication of the virus, other proteins also play roles. For instance, N binds to NSP2 before transcription of viral RNA takes place [129]. Since it involves other proteins, we should therefore expect limitations and potentials in N and M just as we have seen this in S. One such example is the use of Virulence-Inner SDM to compare distant viruses within a family. This is the case when the viruses proteins are essentially very different in sequences and use different receptors. Other examples of the limitation of SDMs can be detected in the results certain animal and viral titration studies. Discussion in further details can be seen in the next subsection.

5.10. A More Accurate Understanding of S Comes with the Study of Other Proteins

The above sections do not show that S does not play any role in infectivity and virulence but, rather, that S does not play the roles in infectivity and virulence that many scientists have envisaged. To be able to gain a more accurate understanding the real role of S, a better understanding of the role of other viral proteins, especially the more abundant major proteins, is necessary.

We have presented evidence to show that S is not able to account for the differences in virulence and infectivity between SARS-CoV-1 and SARS-CoV-2. We have also seen that the viral titers of the two viruses are different with higher titer, implying higher virulence in the case of SARS-CoV-1. While the presence and absence of FCS in SARS-CoV-2 and SARS-CoV-1 respectively could explain the greater virulence of SARS-CoV-1, it does not explain the greater virulence of the latter. The SDMs using N and M provide a comprehensive and coherent explanation that is more reproducible. The SDMs are also able to explain the differences among COVID-19 viruses, even if they are more complex. Much of the problem lies in the fact that many of the viruses have yet to be observed to spread to the human population, and their actual pathogenicity and infectivity among humans cannot be accurately determined. For these, we have to rely on animal models, the use of which is tricky and requires extrapolation. Wrong inferences based on incorrect extrapolations and assumptions can easily be made, as we have seen in the disastrous pre-clinical studies of thalidomide using mice, rats and rabbits [133].

In the case of COVID-19, it was believed that the FCS is responsible for infectiousness and virulence based on an animal model, in which infected and uninfected ferrets were caged together. One group had a few ferrets infected with a wild-type SARS-CoV-2 (WT), whereas the other had a few that were infected with a mutated strain without FCS. The result was that the WT did infect some of the uninfected ferrets, whereas those infected with FCS-mutant did not infect others [112]. Based on this experiment, it is easy to come to conclusion that FCS is responsible for high transmissibility. A picture that contradicts this conclusion was obtained when similar experiments were performed using pangolin-CoVs and Omicron.

In various independent laboratories, uninfected Syrian hamsters were easily infected by those infected with pangolin-CoVs (Pang2017 and Pang2019)

when caged together by contact, though not as easily as via aerosol [75-76,81]. Similar experiments using Omicron yielded comparable results [76-77,114-115]. Furthermore, it was seen that Wuhan-Hu-1 is more easily spread via aerosol, whereas pangolin-CoVs and, to some extent, Omicron were not, even though the latter have been shown to spread easily clinically. Keeping in mind that pangolin-CoVs don't have FCS but Omicron does, these experiments highlight that the issue is more complex than it seems, and it is easy to come to the wrong conclusion without a more complete picture. Adding to the confusion, why does Pang2019 show as much virulence as Wuhan-Hu-1 that is not seen in Pang2017 and Omicron?

A more complete and reproducible answer can be found if the analysis takes the various roles of N, M, and S into consideration. It is obvious that S does play some role in the infectivity of the virus, but it is a mistake to assume that this role is as overreaching as suggested by some. SDMs explain that all thus far know variants are highly infectious as COVID-19 patients shed large quantities of the virus because its hard outer shell M helps prevent virion disintegration caused by salivary and mucosal enzymes before being expelled by the body. While the abnormally hard M ensures a certain high level of spread, higher N PID (ie. Higher levels of N disorder) leads to greater levels of viral replication and viral load (the mechanism has been explained in subsection 5.9), especially in vital organs such as the lungs, that could increase the virulence of the virus and the amount of viral particles expunged from the body, even if the quantity of virus shedding is already high. This is exactly the case with Wuhan-Hu-1 and Omicron. Omicron has a relatively low N Disorder (PID ~43%) and is therefore milder than the other variants, but it is also infectious as it has also low M PIDs (~5.4-5.9). Wuhan-Hu-1, on the other hand, is both highly infectious and more virulent as its M and N PIDs are low and relatively high, respectively. The aforementioned animal model suggests that Wuhan-Hu-1 is relatively more infectious than Omicron. Since both SDMs and clinical studies suggest high infectiousness in the two viruses, the experiment can only be consistent with SDMs and clinical studies if the infectiousness of the two viruses are only relative in differences [71-73,114-115]. We have to be careful when using animal models since extrapolation is necessary when we attempt to link the results to human infections [132].

Furthermore, human infectivity tells only part of the story in the evolution of SARS-CoV-2 and its ancestral strain. We must also keep in mind the infectivity of the SARS-CoV-2 and its relatives in different animal hosts. Furthermore, we must pay attention to data pertaining to virulence including viral titration and cell damage. As virulence can be linked to infectivity using the SDMs and MCC as shown in 5.4-5.6, these factors are important when it comes to interpretation of the results of animal models involving infectivity. We will also see in the next section that the best understanding of the results comes when we use our knowledge of S, N and M in tandem.

5.11. Biological Implications of a More Rigid M

We have seen in Figure 2 that all SARS-CoV-2 related viruses have an extraordinarily hard outer, M. Nearly all CoVs have PIDs of above 8. The exceptions are CoVs associated with burrowing animals. CoVs closely related to SARS-CoV-2 have M PIDs between 4% through 6.3% (Table 2, Supplementary Table). The differences between burrowing and non-burrowing seem small even if statistically significant. A clearer understanding comes, however, if we consider the functions and virion physiology related to M. M is the most abundant viral protein that spans the entire virion. Therefore, even with a few mutations that lowers the disorder of M, the rigidity of the SARS-CoV-2 membrane is likely be strengthened greatly by the sheer abundance of M. This is especially so when the virus is in its native state when M plays the essential role of protecting the virion from external insults.

A question that arises is: If SARS-CoV-2 M is more rigid, how is it able to perform its other functions that requires conformational changes such as viral entry and protein assembly. If we look at Table 2, we see that the minimum M PID seen in all CoVs is around 4%. Obviously, this is the minimum level of disorder that is needed for M to have just enough flexibility in order to perform its other roles that require conformational change. A M PID of 0% theoretically means that the protein is has no

room for conformation changes. Therefore, an M PID of 0 % means that the virus is not functional. The data in Table 2 and Figure 2 seem to reinforce this idea by the existence of the 4% minimum cutoff point. While a lower M PID offers greater protection to the virus in its native state, it could also imply that M could have less efficiency in undergoing conformational changes. We don't, however, know how much impact this has on the efficiency of conformational changes that are necessary for processes such as viral entry and protein assembly. Extrapolating from the efficiency of SARS-CoV-2 (M PID ~ 6.3%) in replication, it is likely not that much, but, again, this is just an extrapolation that has to be confirmed by experimental results.

5.12. A Comparative Analysis of SARS-CoV-2, Pangolin-CoVs, and Laotian Bat-CoV Experiments Using S, N, and M

A more comprehensive analysis can be accomplished when examining M, N, and S in experimental data for SARS-CoV-2, bat-CoVs, and pangolin-CoVs. We have seen that all COVID-19-related viruses are potentially infectious because of their abnormally hard M. We have also seen that Pang2019, BANAL: (Laotian bat-CoV) and Wuhan-Hu-1 are all potentially virulent because of their high N PIDs (~48%), whereas Pang2017 and Omicron are attenuated as a result of their relatively low N PIDs (~44%). These have been largely reproduced. Cells infected with Pang2017 or Omicron have signs of lower viral growth and cytopathic effects [55,74]. Similarly, animal models have shown less severity. In contrast, however, viral titers of cells infected by Pang2019 [80-82] and Wuhan-Hu-1 [75,79] are higher than those infected by Pang2017 or Omicron [55,74-76], just as predicted by the SDMs. In contrast, mice were observed to be severely sickened by Pang2019 [81-82]. We need to keep in mind that this trend is true despite the fact that FCS is found only in SARS-CoV-2 including Omicron. Therefore, the stark differences in the results of the various experiments can only be accounted for when the roles of N and M are considered.

5.13. Evidence of the Potentials and Limitations of S: Viral Entry and Replication

The data for the Laotian bat-CoVs present an enigma [50-51]. It was shown that BANAL S binds to ACE2 in a different manner from SARS-CoV-2 even as the BANAL viruses bind efficiently to human cells. Ironically, even though the N PID of BANAL is close to those of Wuhan-Hu-1 and Pang2019, no severe disease was detected in mice infected by BANAL-236. This seemed inconsistent with what SDMs predicted until the data were studied very carefully. If we inspect the viral titration data, we can see that the viral titers in VERO-E6 cells is high and comparable to Wuhan-Hu-1, but this is not the case in CALU-3 cells, where the difference is larger; it should also be kept in mind that a VERO-E6 cell is of kidney origin, whereas CALU cells are respiratory. This implies that BANAL-236 is more adapted to kidney cells than respiratory ones presumably because of its S structure. We also need to keep in mind that it has been observed that the Laotian bat-CoV S binds to ACE-2 in a different manner [51].

While this serves as evidence that S does play a role in infectivity, it does not show that SDM results are wrong or not reproducible. Instead, it points to the correct way that SDMs should be interpreted. Firstly, the experimental data remind us that SDMs predict *potential* virulence, not necessarily actual virulence, and this potential virulence arises from the high viral load in at least one organ, which is populated with cells that the virus can enter more easily. A second lesson to be learned from the data is that SDMs predict virulence in general, not just humans, since N PID correlates best with highest viral titers among various cell types [55,74-79], and high viral load is associated with organ failures. While human COVID-19 fatality is mainly associated with pulmonary (lung) failures, this may not be necessarily so for other animals. Therefore, SDMs are predicting potential virulence in general, not just in humans.

If there is evidence that S does play some role in infectivity and virulence via viral load, the question then becomes: how easily does S adapt to bind more efficiently? It would seem that greater S fitness may be more easily acquired than many believe. Experiments have shown that COVID-19 viruses acquire greater ability to infect the lungs after passing to humanized mice several times [133-136]. Furthermore, a closer comparison of the two may provide clues. Given the fact that both BANAL-236 and Pang2019 have high N PIDs, why has Pang2019 been shown to be virulent to humanized mice, unlike BANAL-236? Evidently, Pang2019 S is more adapted than BANAL-236. To understand how this is the case, we need to look at the evolutionary differences between the two viruses. If we look at Figure 5B, which is a phylogenetic tree using M, we see that Pang2019 is much more closely related to SARS-CoV-2 than BANAL-236. This implies that Pang2019 was exposed to a similar range of hosts as Wuhan-Hu-1. What is also remarkable is that both BANAL-236 and Pangolin-CoVs, unlike SARS-CoV-2, do not have FCS. It is likely that Pang2019 split off from SARS-CoV-2 to infect mainly pangolins, and as a result lost its FCS while still maintaining much of the rest of its S structure. This may also have implications for Peacock et al.'s FCS-mutant experiment [113] It is possible that FCS-deficient S compensates for its deficit by binding to ACE-2 in a different way over the long run as in the case of Pang2019, and, as we will see, it is easy for S to quickly adapt to the ACE-2 of a particular species or cell type in a laboratory.

It is evident that S has to be at least sufficiently adapted to ACE-2 in order to even be infectious, but in order for SARS-CoV-2 to have any sustained infectivity or virulence, other factors must also come in crucial play, especially the roles of M and N, as seen in the experimental and clinical evidence pertaining to SARS-CoV-1/2. The question then becomes: how difficult it is for S to gain sufficient adaptation to respiratory cells? The earlier section argues that it may not be that difficult. Several studies have shown that COVID-19 viruses can easily acquire adaptability to human respiratory cells in the laboratory very quickly [133-136]. Acquiring abnormally hard M that provides for potentially high infectivity, on the other hand, may not be that easy. It is easy to find CoVs that efficiently bind to S of respiratory cells [101,109,118] or have FCS [101,109,118] or that S easily adapts [108,131-132], but it is difficult to find CoVs with the abnormally hard M, as most CoVs are not intimately associated with a burrowing animal unlike COVID-19 related viruses [4,16-20,53-56] .

5.14. Greater Model Reproducibility and Reliability Come When More Proteins are Considered

The greater reliability and reproducibility of SDMs, in large part, arise from the fact that they take into account the disorder of two major proteins, M and N, which are most abundant in the virion and infected cell respectively. In fact, SDMs become unreliable if M or N is omitted from consideration. SDMs are reliable and reproducible in most cases except in certain occasions when the role of S has to be taken into serious consideration as we have seen in the case of the Laotian bat-CoVs. For this reason, we cannot dismiss S or any other proteins as unimportant. In fact, as we have shown, SDMs become even more reliable and reproducible when S (or other protein) is taken into consideration. This trend is consistent with what we know about the biology of viral replication. Viral replication involves multiple proteins even if there are proteins that play more major roles than others.

5.15. Limitations and Potentials of N, M and SDMs

We have already touched on the limitation of SDMs. We have seen that it cannot account some of the experimental results conducted using BANAL (Laotian bat-CoVs). They are, for example, unable to account for the differences in the the viral titrations of BANAL-236 and SARS-CoV-2 on different cell types (e.g. CALU, COCA, Vero-E6) [51-52,77]. The significance of the differences is, however, needs further investigation as other researchers have shown that SARS-CoV-2 becomes more efficient in replication as several passages are made in each cell type. Nevertheless, this points to the role of S,

which is a limitation of the SDMs. Currently, SDMs involves on the viral shell proteins, which, in the case of CoVs, are the N and M. Given that SARS-CoV-2 has 29 viral proteins and many of them are involved in the replication process [106-109], there will be limitation if only one or two proteins are used to study infectivity, virulence or long COVID. This is, of course, the case in SDMs, which currently use only M and N. In fact, as already mentioned, without either M or N, SDMs would be of limited reproducibility and reliability and would have much difficulty explaining the underlying cause of infectivity and virulence.

Ironically, the question is then: How could M and N account for infectivity and virulence with even a decent level of reproducibility and reliability when the limitation of using only two proteins is considered? The answer could lie in two factors. The important roles of M and N, as seen above, are likely to be partly responsible. Secondly, a hint of an overwhelming importance of M and N can be seen by the massive abundance of the proteins, not seen in other COVID-19 viral proteins. This could also reinforce the idea that the two proteins are playing greater major roles in the functioning of the virus. While the focus of this review paper is on SDMs, N and M, it is not intended to dismiss the importance of S or any other viral protein. This review, however, attempts to underscore the importance of M and N in infectivity and virulence as exemplified by the exercise seen in Figures 6-7. The results seen in Figures 6-7 do not invalidate the role of S, as Hui et al attempted to demonstrate but, rather, suggest that the currently understudied roles of M and N could even overshadow that of S with respect to infectivity, virulence and, potentially, long COVID. Of course, SDMs would become more reproducible and reliable if more viral proteins such as S and NSP7 are considered in the models, but, currently, SDMs have not reach the stage where the roles of more proteins can be incorporated.

6. Pangolin Footprints and Long COVID

6.1. The Long COVID Enigma and Pangolin Footprints

One unusual characteristic that is often found among some COVID-19 patients is long COVID, which is when symptoms persist for weeks, months, or even years after the initial infection [57-58,110-111]. While the cause of long COVID remains largely a mystery, SDMs offer the most logical and plausible explanation yet. We have seen that all COVID-19 related viruses have extraordinarily hard outer shell (M) that is not found in any CoVs except those associated with burrowing animals. We have also seen how SDMs explain that the hard M allows for greater infectiousness of COVID-19, by providing more resistance to mucosal and salivary antimicrobial enzymes, often without greater virulence that is associated with greater N disorder [18-20,52,55-56]. Just as a hard M provides resistance to antimicrobial enzymes, it will almost certainly also provide resistance to other destructive mechanisms offered by other aspects of the host immune system. This is possible as clinical studies have shown that large amounts of the virus tend to remain in the body even after months [110-111]. We will further explore this link by a further examination of the various known aspects of immunology related to this matter as seen in the following subsections.

6.2. Hard M Resistance to Virolysis by Macrophages and Complement System

In order to further examine the role of an abnormally hard M in long COVID, we need to look more closely at how the immune system gets rid of invading foreign particles, especially viruses. Our knowledge of immunology helps us to focus our attention on proteins produced by the complement system and lysosome [135-142]. These enzymes are experimentally shown to damage viruses and viral membranes.

Complement proteins are produced mainly by hepatocytes in the liver, even though they are secreted by monocytes, macrophages, and epithelial cells in the intestines. There are, at least, 30 types of complement proteins [139]. B and T cells, along with antibodies, can alert the complement system in the presence of a foreign matter such as bacteria or virus. Complementary proteins assemble and bind to a protein at the targeted membrane, and the protein complex punches holes in the membrane. Apoptosis occurs in the case of bacteria or infected cells, whereas, in the case of a virus, this is referred to as virolysis. It is at this point in COVID-19 and long COVID that the hard M may make it difficult for the complement system to do its job, as the proteins are likely unable to penetrate the membrane since M spans the entire membrane that covers the virion.

6.3. *Resistance to Virolysis Within a Macrophage May Provide the Virus a Place to Dwell: Possible Reservoir*

A second way that the immune system attempts to get rid of pathogens is to expose them to digestive enzymes [130-131,140]. The macrophage will first engulf the microbe or microbial protein, and upon phagocytosis, the immune system will attempt to digest the particle via lysosomes [138-141]. The problem with this strategy, however, is that many pathogens are somehow resistant to the exposure of digestive enzymes and end up living in the macrophages themselves [140-141]. An ongoing enigma pertaining to long COVID involves the possibility of a reservoir that harbors the virus long after the initial infection of the patient and where the source actually is, since a hard outer shell could prevent the macrophage from destroying the virus and the virus ends up living in the macrophage. The observation of an abnormally hard M, along with our knowledge of immunology, suggests that the first place to look at is none other than the macrophage itself, and current research supports this. Huot et al. [144] found the presence of SARS-CoV-2 in the lungs of patients with symptoms of long COVID, whereas several other research groups have found presence in organs and tissues throughout the body [110-111], which is consistent with the fact that macrophages can be found in nearly all organs in the body [139].

The nature of SARS-CoV-2 should not be confused with that of other viruses such as HIV and HSV. The fact that SARS-CoV-2 has among the hardest outer shell, not just among CoVs but also among all viruses, is a tell-tale sign that the virus is of a different nature from other viruses such as HSV and HIV-2 that are known to hide in places such as the brain only to show up later [109]. HIV and HSV-2 have one of the most disordered outer shells among viruses, unlike SARS-CoV-2. Their highly disordered outer shells help them penetrate and hide in organs, as greater disorder allows for more efficient protein-protein binding. SARS-CoV-2, on the other hand, has one of the hardest outer shells. If it does not have the benefit of a disordered outer shell, how does it hide? The answer has to lie in the mechanism of phagocytosis described above. There is research showing that inflammatory responses may be responsible for long COVID. A hard M and inflammatory responses such as those caused by cytokines are not mutually exclusive [142-144]. Because of the extraordinarily hardness of M, it provides for a unique way for SARS-CoV-2 to hide in macrophages only to appear whenever the opportunity arises. Whenever the virus keeps reappearing, the immune system could respond in such a way that could result also in inflammation caused by cytokines.

6.4. *Granuzymes: A Suspected Mechanism of M Resistance*

While the exposure of destructive enzymes has been shown to act against viruses in the complement system and macrophages, there are also other destructive enzymes available in the immune system. These involve a family of enzymes known as granuzymes. Upon entry of the virus, the immune system will initiate a variety of defensive actions that include Cytotoxic T-Cells and Natural Killer (NK) cells, which secrete substances that could potentially damage viral particles [136-139]. These cells secrete granuzymes in response to a foreign invader. One of the granuzymes is perforin, which binds to plasma membranes and punches holes that allows other granuzymes to enter to cause further damage to the bacterium or infected cell [145]. While current

experimental evidence has shown this to occur in membranes of bacteria and infected cells, there is currently no evidence that it damages viral membranes. Nevertheless, given the known biochemical capabilities of perforin, it can be construed that perforin can potentially damage virion in the same manner, since most animal viruses including SARS-CoV-2 have a protective outer membrane layer to protect themselves. In any case, perforin causes lysis in infected cells, and thereby allows the virus particles to be exposed to the complement system and macrophages. It is also known that, while T-Cells and NK cells secrete perforin, macrophages secrete perforin-2 (PFN2), which is similar to perforin, but it remains unclear if PFN2 affect viral membranes directly [144-145].

6.5. Uniqueness of COVID-19 Strategy of Immune Evasion in Long COVID

There are many mysteries involving long COVID that physicians and scientists are still struggling to solve so that we can come up better treatments. How does SARS-CoV-2 induce long COVID? What are the mechanisms? Is there a reservoir? If so, where is it? As we have seen, SDMs offer specific answers to these questions. While N disorder modulates the amount of virus replicated, especially in vital organs, the unusually hard M provides resistance to antimicrobial enzymes. Therefore, the immune evasion strategy used by SARS-CoV-2 is drastically different from that of viruses such as HIV, HSV, and HCV [4,16-20,65-67], which have high disorder in the other shell that allows such viruses to hide in organs. Not only has no such high disorder been detected in SARS-CoV-2, the virus has one of the hardest outer shell among viruses, not just CoVs. That is why the macrophages offer the virus the opportunity to dwell and hide in them. Indeed, one laboratory showed the presence of the virus in the lung alveolar macrophages of patients who tested negative for the virus in the upper respiratory system [143]. What is even more puzzling is that several other laboratories have observed the presence of the virus in many organs of long COVID patients [111-112]. This raises the question, where is the reservoir? Again, if macrophages are the reservoir, the virus will be present in these various organs since macrophages are found in nearly every organ in the body.

6.6. Long COVID, long SARS and S

Long COVID has been linked to the activation of the immune system, via, particularly, interferons (IFN- γ) and natural killer T-cells (NK cells), that results in inflammation [142,144]. Many scientists point to S as the main underlying cause of the activation. While this postulation is very interesting and plausible, it raises more questions than it answers. For instance, it doesn't tell us the reason why some people get long COVID, whereas, others don't. It doesn't tell us if long COVID arises from a reservoir and, if so, what is the source of the reservoir i.e. the hiding place of the virus. We also need to remember that SARS-CoV-1 has a CoV S protein too. Because the two viruses are relatively closely related (80%), it is likely the S of both viruses are functionally similar including their ability to activate the immune system in similar ways. A conundrum is, however, seen when upon an examination of the differences between long COVID and long SARS. One stark difference is the fact that long SARS was usually associated with severe manifestation of the disease, whereas long COVID could come even with mild symptoms [64]. How could there be such a disparity if the S proteins of the two viruses are likely to be structurally similar and when it has been shown that S activates the immune system [142,144], which is likely to cause long COVID. SDMs offers a novel explanation. SDMs have observed that SARS-CoV-2 has a much harder M than that of SARS-CoV-1 as a result the virus is able to resist the immune enzymes and hide in the macrophage in the case of COVID. We are, therefore, likely to get a better picture when we consider the roles of M, N and S keeping in mind that N also plays a role as greater N disorder could allow greater production of viral particles even in the event of long COVID.

7. Summary and Conclusion

7.1. Reproducibility and Reliability of SDMs: Experimental and Clinical Evidence

Some of the clinical and experimental evidence of the reproducibility and reliability are as follows:

i) The extraordinary hardness of SARS-CoV-2 was seen experimentally by an Australian group, Riddell et al [21]. They discovered that SARS-CoV-2 lasts much longer in the environment away from light than the CoV controls. This is consistent with the detection of an extraordinarily hard SARS-CoV-2 M by SDMs.

ii) The Dutch group, Ogando et al [79] [69] conducted a viral titration of SARS-CoV-2 that was compared with SARS-CoV-1 and found that the viral titers of SARS-CoV-1 are far higher than those of SARS-CoV-2. This is consistent with the SDM pertaining to Virulence-Inner shell disorder, which predicts that SARS-CoV-1 will produce more viral particles in cells and vital organs and, thereby, making the virus more dangerous.

iii) The German group Wolfel et al [29] found that COVID-19 patients shed much more viral particles than SARS-CoV-1 patients. Again SDMs show the greatest consistency in explaining this. How do we explain the discrepancy between (ii) and this discovered mechanism of infectivity? How can we reconcile greater infectivity with lesser virulence and vice-versa? The “S-hypothesis” says that SARS-CoV-2 binds to ACE-2 with 10-1000 greater affinities. If so, how do you account for (ii) and (iii)? SDMs explains that even though SARS-CoV-2 does not replicate as efficiently as SARS-CoV-1 as a result of its lower N disorder. It has a much harder M that is more resistant to the salivary and mucosal anti-microbial enzymes and thus greater amount of viral sheddings occur.

iv) Why did Hui et al's attempt [78] to show that Omicron (S) binds more efficiently to upper respiratory tract (ACE-2) instead shows high statistical correlations with M and N PIDs just as SDMs have predicted [86]?

v) Why is Omicron much milder than previous variants but yet as infectious as other variants? Once again, SDMs provide an elegant explanation as Omicron has lower N PIDs and lower or similar M PID when compared to other variants. The “S-hypothesis” offers an explanation but there are problems with such an explanation as seen in (iv).

vi) Why was Pang2019 found to be virulent in animal models, viral titrations and cell plaques, whereas the opposite was found in Pang2017? The S-hypothesis has no answer especially since all pangolin-CoVs lack FCS. SDMs have already predicted these even before its discovery.

vii) Why do viral titers of the various SARS-CoV-2 variants, SARS-CoV-2-related viruses and SARS-CoV-1 correlate with their N PIDs? Why do attenuation and virulence of the various SARS-CoV-2 variants and SARS-CoV-1 as determined by animal models and cell plaque studies correlate with N PID?

viii) What is the nature of long COVID? Again the S-hypothesis has no answer, whereas SDMs have.

ix) Why are SDMs able to tie the different manifestations of COVID-19 ie infectivity, virulence and long COVID under an umbrella of three closely related concepts? The S-hypothesis, on the other hand, still struggles to understand the role of S in the three manifestations.

There are two factors that determine on how good a model is. These are reproducibility and reliability. Reproducibility refers to the ability of independent laboratories getting similar results, whereas reliability involves the ability to consistently explain and predict the results [146-147]. Therefore, a model that is unable

to be replicated in different independent laboratories is unreproducible, whereas, a model that is unable to explain a result is unreliable. (i)-(ix) summarizes both the reproducibility and reliability of SDMs. We would argue that there is definitely evidence of both as we have seen even though, as we have seen, there are certain occasions where other proteins such as S, have to be involved in the explanation.

While the experimental work of Riddell et al is crucial in providing evidence that the abnormally hard M of SARS-CoV-2 is likely to provide greater protection to the virus, in contrast to other CoVs, there is more that could be done. For instance, there are a variety of anti-microbial enzymes found in the mucus and saliva but we know only how they in general can damage viruses. We still don't understand which specific enzymes can damage or kill viruses SARS-CoV-2 or CoVs [22-28]. Experiments need to be done on various CoVs with different levels M PIDs and various enzymes [22-28] so that we can have a better understanding of the mechanisms involved.

7.2. *Links Between Virulence and Infectivity*

An important part of reproducibility and reliability of a model or paradigm is its ability to explain certain results or certain phenomena. We have seen how SDMs are not only able to explain virulence and infectivity but also are able to link infectivity and virulence. The two manifestations are related as virulence involves the ability of the virus to infect cells in vital organs such the virus is able to overwhelm vital organs especially the lungs, whereas infectivity pertains to virus' ability to leave the body to infect the cells of other hosts. We can see that the two manifestations are related but how are they exactly related is complex. A conundrum is quickly seen: Why is SARS-CoV-2 less virulent than SARS-CoV-1 but is more infectious? How did the viruses accomplish this? The S-alone hypothesis is unable to provide a clear answer without further complications and questions (see (iv) in 7.1 and MCC), whereas the SDMs have coherent and clearcut answers to this. According to SDMs, when the virus have higher N disorder (N PID), the virus is able to replicate more quickly because greater disorder at N provides for more efficient protein-protein/RNA/DNA/lipid binding and therefore more efficient and rapid replication. If that is the case, then why is SARS-CoV-1 less infectious than SARS-CoV-2 since MCC entail transportation of much of the viral particles to the nasal region to be expelled ? Again, SDMs have a novel answer and it has to do with the much harder outer shell (lower M PID) found in SARS-CoV-2 and related virus. This abnormally hard M protects the viral particles from the onslaught of anti-microbial enzymes found in the mucus and saliva even if all SARS-CoV-2 variants have lower N PIDs i.e. less efficiencies in replication of viral particles. This is consistent with Wolfel et al's clinical data showing that COVID-19 patients shed much larger amount of virus. As we can see, a more correct approach comes with a coordinated understanding of the roles of M, N and S.

7.3. *Pangolin Footprints in the Evolution of COVID-19*

The phrase "pangolin footprints" refers to a set of molecular signatures that were left behind by the intimate evolutionary interactions COVID-19 ancestral strains had with pangolins. These signatures that involve the abnormally hard M and the trend towards lower N disorder arose from the pangolins' burrowing habit, which entails a harder outer and often inner viral shell to facilitate oral-fecal transmission via buried feces. These features or pangolin footprints are clinically manifested in the symptoms, infectiousness, and attenuation/virulence of COVID-19. A review of current knowledge of immunology indicates that it is also manifested as long COVID. The abnormally hard M that facilitates greater infectiousness by being resistant to antimicrobial enzymes in the saliva and mucus could also resist attempts by the immune system to get rid of it in other ways, thus leading to long COVID.

7.4. *Clues Pointing to Pangolin Footprints in the Evolution of COVID-19*

A unique and highly unusual property that involves all examined SARS-CoV-2-related viruses is its hard outer shell (low M PID). This property is very rarely found even among the CoV family. What is the evolutionary origin of this peculiar characteristic? A retrospective search of our disorder database of CoVs provided us with a clue when it was found that very few CoVs have such exceptionally hard M and those found were associated with a burrowing animal such as rabbits. Furthermore, it can be seen that phylogenetic trees using M have a much closer relationship to SARS-CoV-2 than previous phylogenetic trees have shown. How can this be even possible when RaTG13 has 96.4% to SARS-CoV-2, whereas pangolin-CoVs have approximately only 90% similarity to SARS-CoV-2? What is even more puzzling is that one of our trees shows that Omicron has a closer relationship to pangolin-CoVs than to the other variants. The answer has to do with the fact that phylogenetic algorithms do not handle recombinations well and M may be the best choice for phylogenetic studies since M is abnormally structured (low disorder), which means that it is likely to be highly conserved. Secondly, many scientists have been looking for an intermediary animal host for SARS-CoV-2 to no avail. It has obviously not occurred to many scientists that there is no intermediary animal host is likely because the ancestral virus has entered and re-entered the human population and other animal populations over a long time of time with a primary or secondary reservoir being pangolins. This could explain how all SARS-CoV-2 related virus have unusually hard M and SARS-CoV-2 is highly infectious not just to humans but also to a wide range of animals. It takes time for the virus to become highly adapted to such a wide variety of animals. All these present clues of a more unique evolutionary relationship between pangolins and SARS-CoV-2 that needs to be further research as there are hints that the resulting characteristics are related to the behaviors and clinical manifestations of the virus.

7.5. Greater Reproducibility and Reliability When M, N and S are Used in Coordination

There is thus far no current review article based on updated data attempting to demonstrate the reproducibility that S is the main protein or sole protein responsible for the infectivity or pathogenesis, especially when compared to other viral proteins such as M and N. As we have shown, there is indisputably important clinical and experimental evidence that shows that S cannot account for the difference in virulence and infectivity between SARS-CoV-1 and SARS-CoV-2, even though there is definitely some evidence of its role in modulating infectivity including antibody evasion via S mutation. The behaviors of N and M via SDMs can account for much of the clinical and experimental results and are highly reproducible even to the smallest details. There are important fundamental biological reasons for this. While S is important for viral entry and antibody recognition [109], it is not the most abundant protein. N and M are the most abundant proteins in the infected cell and virion, respectively [109].

Furthermore, viral entry is only the initial, albeit highly important, step in the multistep process of the virus life-cycle. We have, however, also seen that the roles of S in infectivity and virulence become clearer and more consistent when we examine other proteins, especially M and N, alongside S. The interplaying roles among the various proteins have to be considered before we can fully understand the actual nature of these proteins and their impacts on infectivity and pathogenesis. Without S's greater adaption to ACE-2, there would be inefficient viral entry, if any, but without the hard M and varying N disorder there would not be any highly sustainable infectivity or the diverse levels of virulence respectively seen in COVID-19.

Also, as mentioned, S alone cannot account for the differences in infectivity and virulence between SARS-CoV-1 and SARS-CoV-2 without further questions, given current clinical and experimental data. N and M, on the other hand, do provide a coherently novel explanation that should be further explored. It is also worth noting that many laboratories have shown that it is easy for S to adapt to the ACE-2 even within respiratory cells [133-135], but it is extremely difficult to find CoVs with such a hard M as in the case of SARS-CoV-2-related viruses [4,18-20,52-54]. Acquiring such hard M which is necessary for potential high infectivity had to take its time to evolve from the

interactions with a burrowing animal, i.e. pangolins, and none of the high COVID infectivity we have seen would have been possible with this crucial evolution. Furthermore, it has been argued that nothing is unique about SARS-CoV-2, including its FCS, since it is not difficult to find CoVs with similar properties. In our dataset, we are, however, unable to find CoVs with such an abnormally hard M among CoVs not associated with a burrowing animal, and there are very few CoVs associated with a burrowing animal, if we don't count COVID-19-related viruses.

7.6. Long COVID

Furthermore, S is not able offer a plausible hypothesis for the cause of long COVID. The cause of long COVID has thus far remained a complete mystery, which is a great hindrance for the search for more effective treatments. The M and, to a much smaller extent, N via SDMS, however, offer an elaborate and highly plausible explanation using our current knowledge of immunology on the cause of long COVID: the hard outer shell of the virus is likely to make it difficult for macrophages, T-Cells, and other such entities to get rid of the virus. As a result, there may be plenty of opportunities for the virus to dwell in the macrophages, which are a likely reservoir.

7.7 Clues for Further Research

We have seen that SDMs offers a novel coherent that links virulence and infectivity with N and M via protein intrinsic disorder. Both experimental and computational evidence of the reliability and reproducibility [146-147] of SDMs as applied to COVID-19 is covered. We have tried to show that SDMs using N and M are by and large reproducible when experimental and clinical data especially from other laboratories are scrutinized. The reason for reproducibility and reliability of M and N can arguably be traced to the abundance of the major proteins and their important roles in the replication process. The role of M in infectivity is based on the observation of an abnormally hard SARS-CoV-2 M using AI. Interestingly, phylogenetic study of SARS-CoV-2 related viruses points to a closer relationship between pangolin-CoVs and SARS-CoV-2. This has not been shown in any other phylogenetic study using other proteins or entire genome. While evidence of the reproducibility and reliability of SDMs as applied to COVID-19 using N and M has been presented, further experimental and clinical research is needed to reaffirm their reproducibility and reliability [146-147].

Even though M and N are the most abundant proteins in the virion and cell respectively, there are 29 CoV viral proteins, of which many are also involved in replication and virulence. For this reason, we have tried to show that much of the limitations of COVID-19 SDMs arise from this fact. A solution would be to include more proteins such as S and NSP7 as discussed. This is where further research is also necessary since the current SDMs incorporate only N and M.

While we tried to argue using the existing framework of SDMs and current knowledge of immunology that SDMs as applied to long COVID is promising, the models as currently applied to long COVID are still preliminary or at its infancy and, therefore, rely on circumstantial evidence. Given that long COVID is still largely a mystery, it is imperative that long COVID be further researched into using novel means including SDMs.

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Conflicts of Interest: G.K.M.G. is a research scientist and the owner of Goh’s BioComputing, Singapore. He has written a book, “The Viral Shapeshifters: Strange Behaviors of HIV and Other Viruses” [4]. The authors declare no other conflict of interest.

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