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Opinion

Nano-targeting lessons from the SARS-CoV-2

I.R.S. Ribeiro a, b, 1, R.F. da Silva a, 1, C.P. Silveira a, 1, F.E. Galdino a, b, M.B. Cardoso a, b, *

a Brazilian Synchrotron Light Laboratory (LNLS), Brazilian Center for Research in Energy and Materials (CNPEM), 13083-970, Campinas, Brazil
b Institute of Chemistry (IQ), University of Campinas (UNICAMP), 13083-970, Post Office Box 6154, Campinas, SP, Brazil

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The lack of targeting efficacy has frequently led functionalized nanoparticles to accumulate in unwanted cells and tissues while boosting toxicity-related effects. Conversely, viruses are natural nanoparticles that precisely and responsibly interact with the biological machinery through an effective-driven fashion. This interaction is enhanced by a meticulous spatial arrangement which results in a quasi-crystalline distribution of proteins on the viruses' surface. Amidst the COVID-19 pandemic, we propose to look at the SARS-CoV-2 nanoscale viral scaffold as an example of a highly-ordered architecture that must inspire and tailor the production of targeted synthetic nanoparticles.

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Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic has taken the world’s attention due to overwhelming infection and mortality rates. While COVID-19-related research advances at remarkable speed, all other scientific areas have suffered from social distancing or lockdown restrictions. Therefore, the scientific community must use this moment to reflect and understand what structural characteristics give the SARS-CoV-2 such a high targeting and infectious efficiency [1, 2].

Over the past decades, science has advanced to a point of astonishing control over materials at the nanoscale and nanomedicine has developed sophisticated platforms able to improve therapeutic outcomes significantly [3]. However, one of the main challenges in the field remains the design of successful targeting strategies [4]. Many of the currently available nanoparticle formulations undergo non-specific interactions that result in random drug delivery and cellular internalization profiles. These interactions divert nanoparticles from their intended path, generate a series of off-target effects and leave them more vulnerable, for example, to phagocyte clearance. Viruses, in turn, are considered as successful examples of targeted nanoparticles that have been refined through evolution.

For instance, SARS-CoV-2 uses its structural spike (S) glycoprotein specifically to mediate its entry into host cells [5, 6]. This protein is homogeneously distributed on its surface as part of a well-defined geometry and symmetry [7]. Such surface homogeneity is not observed in laboratory-made nanoparticles, since standard functionalization methods lead to a non-homogeneous surface coverage which likely hinders the specific interaction between active groups and their receptors. Scientists have looked to viruses for inspiration for some time [8–11], however, to our knowledge, most strategies only benefit from either viral topographic features or specific receptor-mediated interactions. Therefore, we suggest the key to improve targeting strategies may lie in structural features that bring together the meticulous viral surface organization and responsive interactions with cell receptors.

Here, we shed light on the SARS-CoV-2 architectural characteristics as an example of a highly-efficient targeted nanoparticle, establishing an analogy with the current challenges regarding synthetic targeted nanomaterials.

SARS-CoV-2 targeting efficiency

SARS-CoV-2 (60–140 nm) provides a nanoscale scaffold that can be explored as a model for studying and developing synthetic nanoparticles for biological applications [12, 13]. Before establishing an analogy with engineered nanomaterials, it is necessary to understand the viral structure and its interactions with the host
machinery. The SARS-CoV-2 structural spike (S) protein is homogeneously distributed on the virus surface and plays a central role in viral tropism through selective binding to the human membrane receptor angiotensin-converting enzyme 2 (ACE2) [2,14]. The nose is the initial site of airway SARS-CoV-2 infection and serves as a reservoir for the virus to spread across the respiratory tract [2].

Notably, ACE2 receptors are specifically harbored on the motile cilia of nose epithelium cells [15], which gives the virus considerable advantage. First, the cilia length is about 50-fold the virus size (~5 μm vs. ~100 nm) and represents a substantially large surface area containing receptors for virus attachment (Fig. 1) [16]. Second, this virus–cilium interaction impairs proper cilary function in later disease stages, inhibiting the mucociliary clearance that could prevent further viral infection [15]. Importantly, SARS-CoV-2 presents a 10-fold stronger binding affinity for ACE2 than SARS-CoV, although both viruses share 76% of the S protein genetic sequence [17]. In coronavirus, the S glycoprotein, a homotrimer comprised of two domains (S1 and S2), presents a conformational flexibility that is used both to evade immune surveillance, in which the receptor binding domain (RBD) is hidden and less likely to be found by defense cells (lying down conformation), and specifically to bind to ACE2 receptors, in which the RBD is exposed to the environment (standing-up conformation). SARS-CoV RBD is predominantly found in the “up” state, whereas the SARS-CoV-2 RBD is predominantly found in the “down” state. Even so, SARS-CoV-2 is able to maintain high infectivity rates. The reason is that, upon activation, the S protein radically changes its conformation and exposes the RBD to bind to ACE2 receptors with incredible affinity. These changes involve the shedding of S1 enabled by host proteases at the cell surface that cleave the S1/S2 junction – such as the serine protease TMPRSS2. This cleavage is possible due to a four amino acid insertion (PRRA) in the S1/S2 region that is particular to the SARS-CoV-2. This results in the exposure of a conserved C-terminal sequence that is known to bind to neuropilin co-receptors, thus creating an additional binding site for virus entry [18,19]. Upon cleavage, S goes from what is called the pre-fusion to the post-fusion conformation. It was found that the S proteins rotate freely around their stalks when in the pre-fusion conformation. This rotational freedom is not usually found in other coronaviruses and is associated with the sparser S protein density observed in the SARS-CoV-2. Importantly, this rotational freedom may facilitate the engagement with ACE2 receptors since these are dimers and bind to two RBDs at a time. Moreover, pre-fusion S are spaced by 15 nm average distance between each other, which may also be key for the ACE2 binding [20]. Furthermore, SARS-CoV-2 S proteins are densely and homogeneously decorated with several kinds of N-linked glycans composed of complex-type and oligomannose-type glycans that ensure adequate folding of the S protein, modulate the accessibility to ACE2 and often act as a physical shield by helping the virus to evade immune system, since the glycans, mostly belonging on the host cell itself, are rarely recognized as invaders [5,20,21]. Therefore, SARS-CoV-2 can be considered as a complex nano-structure with a remarkably effective protein-receptor pair that is highly sensitive to in loco stimuli to complete its internalization.

The nanostructured viral symmetry

Viruses are highly ordered biological structures. They display a surface organizational level where repetitive units of specific protein domains are located at almost-crystalline distances [7]. The viral protein arrangement is an example of natural self-assembly and results in nano-structures with characteristic geometries. Therefore, viruses are classified within mathematical models that describe their symmetries [7]. This highly symmetric architecture allows viruses to build up their structures by coding the maximum number of protein copies from the minimum genetic information – a principle that Crick and Watson called genetic economy [7]. Proteins adopt quasi-equivalent positions within the viral geometric constraint and repeat this configuration throughout the structure [22], forming what resembles an almost artistic mosaic-like composition.

This meticulous spatial arrangement enables the occurrence of multivalency where multiple ligand copies interact simultaneously with various cellular receptors [23]. This phenomenon turns a number of weak interactions into a strong binding event which increases the internalization probability [23]. Instinctively, the scientific community usually illustrates synthetic functionalized nanoparticles with homogeneously-distributed ligands on the surface. However, commonly-used functionalization protocols lead to a non-homogeneous ligand distribution [24]. Therefore, the nanoparticle surface displays areas with much higher ligand density than others, as well as areas with no ligand at all [24]. This lack of surface homogeneity is, perhaps, the most striking difference between laboratory-made nanoparticles and viruses. Although it is not completely clear to what extent nanoparticles can benefit from ligand arrangement homogeneity and/or symmetry, some works suggest that an optimal interligand distance can result in stronger protein-receptor interactions, especially when one of them is a dimer/trimer [25,26]. We can envisage possible consequences of a random distribution regarding cellular recognition and internalization. A non-homogeneous ligand spacing may impair the advantage of multivalent interactions, since they are highly sensitive to the ligand density and distancing [25,26]. This, in turn, likely decreases the effectiveness of nanoparticle uptake through specific mechanisms. Naturally, the binding affinity constant plays a pivotal role and, for some protein-receptor pairs, a single binding event may be enough for the cell to internalize the nanoparticle. Nevertheless, the viral homogeneously-arranged protein distribution statistically favors a successful binding encounter when all particle rotational degrees are taken into account. For SARS-CoV-2 specifically, this probability is tremendously enhanced by the aforementioned presence of ACE2 on the cilia of nasal epithelium cells by the S protein rotational freedom, since both are flexible enough to assume spatial orientations that favor multiple binding events. For engineered nanomaterials, the probability of a successful anchoring event is hindered by a noticeable chance that the nanoparticle encounters the surface portion of the cell that presents no active binding groups, which consequently results in no recognition (Fig. 2a). It is worth mentioning that viral particles are all quasi-identical copies (as long as mutation is discarded). Thus, the evolution-optimized protein arrangement is present on every particle and maximizes the chances of molecular recognition. Conversely, current functionalization methods result in nano-formulations that are collections of “one-of-a-kind” particles and compel researchers to adopt the concept of an “average” nanoparticle that is, in fact, illusive.

Inspired by nature

SARS-CoV-2 can be simplified to a nanoparticle that reaches its target almost without obstacles and interacts with its target receptors with remarkable affinity. We can then endeavor to translate this extremely targeting-effective example to the nanomedicine context. Although this exercise seems trivial, we must thoroughly take into account the viral structure and overcome synthetic and functionalization barriers to maintain the bio-functionality of active groups on the surface of engineered nanomaterials. Thus, one of the most challenging tasks to mimic natural entities is related to the precise control of the surface organization. SARS-CoV-2 pre-fusion S protein, as mentioned, presents
an average distancing of 15 nm and benefits from a rotational freedom that confers the necessary flexibility to assume orientations that favor efficient binding to ACE2 dimers. To mimic the viral organizational level, nanomaterial designers need to surpass current methods and couple precise synthetic approaches with surface functionalization strategies that allow the control of average-distancing between bio-active groups. This would naturally improve intra-batch reproducibility (i.e. homogeneity among particles), which we see as a key factor to advancing targeted nanomedicine. The translation of surface patterning techniques (e.g. DNA origami)\cite{27,28} to the nanomaterials surface represents a promising alternative. Likewise, microfluidic devices offer impressive control over nanomaterials’ physico-chemical properties and have been extensively used in nano-synthesis \cite{29}. Similarly, it is highly relevant to highlight that these laboratory-synthesized nanoparticles must maintain their colloidal stability and avoid non-specific adsorption of components that may divert them from the intended target \cite{30,31}.

It is clear that the fine control of surface arrangement could uplift nanomaterial active targeting strategies, which so far have failed to achieve regulatory levels. Another key concept is that bio-logical events are highly selective and most of them are part of a “cellular responsive” mechanism. It is worth recapitulating that the SARS-CoV-2 S protein undergoes dramatic conformational changes to enable receptor binding \cite{5} and viral active groups suffer specific on-target modifications (e.g. S1 shedding) in response to cellular activation. These changes characterize a natural example of what we refer to here as “responsive targeting”. Note that by “responsive targeting” we specifically refer to receptor-mediated targeting in which the bio-active group suffers in loco modifications triggered by the cell to complete the interaction. This concept differs from the stimuli-responsiveness usually applied in drug delivery, in which nanomaterials respond to local stimuli (e.g. pH, temperature) to release their contents \cite{32}. In the case of SARS-CoV-2, this “responsiveness” is essential for the viral tropism, since the RBD is exposed only when it finds its target, keeping the domain safe from immune recognition. Moreover, the modifications on the S protein enable it to bind co-receptors that favor viral entry. It is unclear if protein-functionalized nanoparticles require any co-receptor regulation or structural changes (Fig. 2b) to be specifically recognized and if bio-conjugation techniques impair this flexibility. Therefore, the use of glycans and other post-translational modifica-

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**Fig. 1.** Schematic representation of nanoscale-driven interactions of SARS-CoV-2 with the cellular machinery. SARS-CoV-2 interaction with micrometer-long cilia of the nose epithelial cells (left) and details of the remarkably strong binding between the virus S protein and ACE2 cell receptors (right).

**Fig. 2.** (a) Illustration of non-homogeneous distribution of active groups on the nanoparticle surface, which decreases the probability of specific cell recognition events (left) vs. homogeneous arrangement of active groups, which enhances the chances of a successful binding encounter (right). (b) Representation of a protein conformational change required to enable cellular recognition.
tions to decorate bio-functionalized nanoparticles may be crucial to grant the proteins conformational flexibility required to bind their receptors selectively. In summary, the translation of viral targeting efficiency to nanomaterial design must unify the surface arrangement and architecture with the rationally-designed ability of the nanomaterial to perform on-site modifications of its active groups (proteins) so to increase the efficacy of receptor-mediated interactions and consequently raise cellular internalization rates. We believe this may be the passport to the development of successful targeting strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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