Phospholipids dock SARS-CoV-2 spike protein via hydrophobic interactions: a minimal in-silico study of lecithin nasal spray therapy

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Abstract Understanding the physical and chemical properties of viral infections at molecular scales is a major challenge for the scientific community more so with the outbreak of global pandemics. There is currently a lot of effort being placed in identifying molecules that could act as putative drugs or blockers of viral molecules. In this work, we computationally explore the importance in antiviral activity of a less studied class of molecules, namely surfactants. We employ all-atoms molecular dynamics simulations to study the interaction between the receptor-binding domain of the SARS-CoV-2 spike protein and the phospholipid lecithin (POPC), in water. Our microsecond simulations show a preferential binding of lecithin to the receptor-binding motif of SARS-CoV-2 with binding free energies significantly larger than $k_B T$. Furthermore, hydrophobic interactions involving lecithin non-polar tails dominate these binding events, which are also accompanied by dewetting of the receptor binding motif. Through an analysis of fluctuations in the radius of gyration of the receptor-binding domain, its contact maps with lecithin molecules, and distributions of water molecules near the binding region, we elucidate molecular interactions that may play an important role in interactions involving surfactant-type molecules and viruses. We discuss our minimal computational model in the context of lecithin-based liposomal nasal sprays as putative mitigating therapies for COVID-19.

1 Introduction

Over the last century, humanity has been threatened by several deadly viruses including Spanish flu, SARS Coronavirus (SARS-CoV), Influenza A (H1N1, H5N1), Ebola, Middle East Respiratory Syndrome (MERS-CoV), Zika, and recently Coronavirus disease 2019 (COVID-19 or SARS-CoV-2) [1–3]. To date, the total number of COVID-19 positive cases are in the order of millions according to the World Health Organization [4]. Due to its rapid transmission and high rate of mortality, efforts from different disciplines are being put together in the global endeavour of tackling the Coronavirus pandemic.

The molecular structure of SARS-CoV-2 is formed by a lipid membrane, nucleocapsid proteins, and spike proteins, which together shield the RNA genome of the

virus. It has been shown that spike proteins play a key role in the virus fusion and entry [5], hence they have become one of the key targets for drug design and vaccine development [6–9]. SARS-CoV-2's spike protein is formed by two subunits, denoted by S1 and S2. The S1 subunit binds through its receptor binding domain (RBD) to the ACE2 (Angiotensin-converting enzyme 2) on the cell membrane surface of the lung. On the other hand, the S2 subunit mediates the fusion of the virus with the human host cells [10].

SARS-CoV-2's spike protein binds to ACE2 human receptor with approximately 15nM affinity which is higher than the affinity of SARS-CoV to ACE2 [11] and it leads to increased virulence of COVID-19 [12–14]. The protein and ACE2 can interact via hydrogen bonding, hydrophobic and electrostatic interactions [15–17]. There have been numerous attempts to design or repurpose drugs that would inhibit the binding of the spike protein to ACE2 [18–24]. However, the underlying physical principles that drive these interactions

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are yet poorly understood. To explore novel and potent therapies, a fundamental understanding of the interplay between molecular forces involving the spike protein, potential inhibitors and of course the surrounding aqueous medium, is critical.

Numerous molecular dynamics studies are nowadays aiming to discover potential drugs that could serve as inhibitors or blockers for SARS-CoV-2 [25–28]. It is well established that the binding affinity for drug-candidates is modulated by a subtle balance of interactions involving hydrogen bonding, electrostatics and hydrophobic effects. A class of molecules that have received much less attention in this regard are surfactants which are essentially “soapy” molecules that have hydrophobic and hydrophilic parts. In the field of virology, the importance of surfactants is not completely unprecedented since phospholipids have been employed as carriers for drug molecules in the treatment of several viral diseases [29–31]. Interestingly, phospholipids have been used in treatments to inhibit viral infections [32], to regulate a respiratory viral infection, and to regulate the innate immunity by means of preventing inflammatory processes that result in reductions in gas exchange within the alveolar compartment [33]. Moreover, phospholipids have been shown to trigger the formation of nanofibers, which can bind the envelope of SARS-CoV-2 through electrostatic interactions, and inhibit the virus to enter the host cell [34]. It is also well appreciated that one of the main sources of pulmonary disease within the context of pulmonary viral infections, is the depletion of the surfactant molecules at the air-water boundary in the lungs [35]. Moreover, it has been suggested that, in addition to the well-known function of lowering surface tension at air-liquid interphase, the pulmonary surfactant also possesses host defense and immunological capabilities as a primary airway defense barrier [36,37]. This has led to propose surfactants in the context of putative atoxic, aerosolized drugs [36].

Very recently, intranasal vaccines have been realized as treatments to prevent and curtail the pathology of SARS-CoV-2 in mice [38] and ferrets [39]. Such results have suggested the usage of nasal sprays in COVID-19 therapies in humans [40–42], which is an open area of research. Therapeutic nasal sprays are also successfully used by patients of inflammatory diseases (e.g. rhinitis, sinusitis, conjunctivitis) that affect mucous membranes that are key targets of SARS-CoV-2 [43–45]. Phospholipids are the main constituents of a commercially well-known product called lecithin, which is biodegradable and it can be extracted from both vegetable oils (e.g. soybean) and animal tissues (e.g. eggs) [46]. Therapeutics based on lecithin as principal component (e.g. nasal spray and inhalation solution) have been shown to be effective in the treatment of various health disorders, such as allergic rhinitis [43], rhinitis sicca [45,47], sinunasal symptoms [48], and care after tracheostomy [49]. Chronic respiratory disease e.g. allergic rhinitis were associated with worse clinical outcomes in patients with SARS-CoV-2 [50]. It remains an open question whether

the usage of such nasal sprays could be beneficial in the context of the current pandemic, in particular whether an interaction between supplemented lecithin and SARS-CoV-2 spike protein could be provoked within the mucous lining fluids covering the airways in a clinically-relevant extent. Therefore, given all these connections between the pulmonary surfactant system, SARS-CoV-2, and lecithin therapies, an important question is how does SARS-CoV-2 spike protein interact with lecithin in water solution at the single molecule level?

Herein, we use microsecond timescale molecular dynamics simulations to investigate the interactions that the RBD of SARS-CoV-2 forms with an amphiphilic molecule, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). POPC is a phospholipid constituent of the pulmonary surfactant [51], lipid vesicles [52], and lecithin. We show that lecithin POPC binds preferentially to the receptor-binding motif (RBM) –the hotspot for the binding to the human ACE2– of the spike protein RBD, and that this binding is driven by the formation of non-polar i.e. hydrophobic contacts. These findings are complemented by examining the energetic and entropic contributions to the binding free energy of POPC to the RBM. Furthermore, lecithin significantly alters the secondary structure of the spike protein which is strongly modulated by the concentration of lecithin. Notably, all these processes are accompanied by a significant change in the water local density at the vicinity of the RBM interface. Our molecular-dynamics simulations provide a minimal computational model to address some of these questions in a quantitative manner. These results may help to elucidate the molecular interactions of phospholipids that may interfere with the activity of SARS-CoV-2 but also other viruses [30,31].

Note that the role of water and hydrophobic interactions is not unprecedented given its ubiquitous role in biophysical interactions [53–55] and within the context of our work, viral inhibition. Specifically, recent works have shown that water plays an important role in the inhibition of SARS-CoV-2 via hydrophobic interactions by CB6 antibody and EK1C4 inhibitor [56,57]. Interestingly, the role of hydrophobicity and hydrogen bond networks have also been implicated in the interactions of both Zika and HIV viral membranes constituted by POPC bilayers [58,59].

The rest of the manuscript is organized in the following manner. We start discussing the structure and chemistry of the SARS-CoV-2 receptor binding domain and the lecithin molecule in Sect. “Simulated systems”. After giving the computational details of the system buildup and molecular simulations in Sect. “Computational methods”, we move on to discuss our main findings in Sect. “Results”. Our results summarize the fluctuations in the RBD radius of gyration, RBD-lecithin contact maps, and distributions of water molecules in the hydration shell of the protein. Finally we conclude and present a brief summary of our results in the “Discussion” section.

2 Simulated systems

In this section we introduce and discuss physico-chemical properties of the system in which we focus our study, namely the SARS-CoV-2 receptor binding domain (RBD) and lecithin in water.

2.1 SARS-CoV-2 receptor binding domain

Figure 1a illustrates the molecular structure of SARS-CoV2 RBD monomer (multi-colour) bound to the human ACE2 receptor (green) [27,60]. A zoomed-in view of the RBD molecular structure is displayed in Fig. 1b with the RBM highlighted in red. The residue sequence and its cryo-EM structure of spike glycoprotein's RBD were adopted from Refs. [27,60]. In order to dissect, and better interrogate the protein-lecithin interactions we divided the RBD into non-overlapping zones. Figure 1b shows the five different regions in different colors in the protein structure labelled as Zone 1 (orange), Zone 2 (cyan), Zone 3 (yellow), the receptor-binding motif RBM (red) and Zone 4 (purple). The RBM is the largest domain and carries the residue sequence from Asn439 to Gln505 which has been shown to interact with human ACE2 receptor.

2.2 Lecithin

While it is well known that surfactant-protein interactions play an important role in biological contexts, the underlying physical forces that drive these processes remain poorly understood [61]. Here, we focus our efforts on the phospholipid POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine $C_{42}H_{82}NO_8P$), which we will refer throughout the paper as lecithin, a biodegradable, essential phospholipid in cells. Lecithins are ubiquitous in mammalian organs, they help to build the largest choline reservoir and they are found in the bile [62], and more importantly in the alveolar surface in the lung [63,64]. Lecithin's chemical structure is shown in Fig. 2a. It is a surfactant consisting of a glycerol backbone sterified in positions 1 and 2 with a palmitic and an oleic acid, both constituting the hydrophobic non-polar tail able to interact with non-polar residues. The position 3 of the backbone is linked to a phosphate group which is bonded to a choline group, forming the hydrophilic polar head of lecithin.

Lecithin is amphiphilic and therefore has potential to interact with the spike protein using a combination of both polar and non-polar interactions. In fact, the hydrophilic-lipophilic balance (HLB) of lecithin ranges from values to 4 ± 1 to 10 ± 1 [65]. Thus, it lies in the range of w/o (water in oil) emulsifying agents and also of wetting spreading agents. This is a crucial property that makes lecithin POPC an ideal candidate to act as a molecule targeting SARS-CoV-2, since one will need both polar and non-polar parts, the former related to the polar amino acids of proteins, whereas the latter involves the protein hydrophobic regions. Moreover, at large concentrations lecithin can aggregate into micelles

Fig. 1 Structures of SARS-CoV-2 spike protein receptor binding domain (RBD) and Angiotensin-Converting Enzyme 2 (ACE2). **a** Illustration of the X-ray molecular structure of SARS-CoV2 spike protein receptor binding domain (RBD, multicolor) and human ACE2 receptor (green) [27,60]. **b** Zoomed view of SARS-CoV-2 RBD, where different colours indicate different zones selected to interrogate the RBD-lecithin interactions at different solvent exposures. The red highlighted part is the receptor-binding motif (RBM) of the protein. **c** Amino acid sequence corresponding to the molecular structure of SARS-CoV-2 RBD with the different zones highlighted in different colors. The red dots below few residues indicate those amino acids involved in the interaction with the human ACE2 receptor

[66] which have been employed in the design of nanoparticles coating various drugs.

3 Computational methods

To investigate the structural and dynamical behaviour of SARS-COV2 RBD protein in water and concentrated POPC solutions, all atoms molecular dynamics (MD) simulations were conducted using the multi-GPUs version of the open-source package GROMACS 2020 [67,68]. Adopting a high-throughput approach, we ran multiple simulations in parallel on several compute nodes of the recently installed Marconi100 at CINECA, a world-class European Tier-0 system for high-performance computing (HPC). In all these simulations, we used the OPLS-AA [69] force field together with the SPC/E water model [70].

The choice of OPLS-AA-SPC/E combination was based on various previous benchmark studies done on the assessment of biomolecular force fields and

Fig. 2 Lecithin: chemical structure and interaction dynamics with SARS-CoV-2 RBD. **a** Molecular (top) and chemical (bottom) structure of a lecithin POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) molecule. The bottom figure highlights its polar (hydrophilic) and non-polar (hydrophobic) chemical groups. **b** Snapshot of one initial condition of the simulation setup at, showing the receptor binding domain of SARS-CoV-2 spike protein in the lecithin solution, with water molecules depicted as

transparent gray spheres. **c–d** Snapshots taken at times larger than $1 \mu s$ for two different values of the lecithin concentrations corresponding to $N_L = 10$ **c** and $N_L = 15$ **d** number of lecithin molecules in the simulation box. For the sake of better visualization, we do not include the water molecules in panels C and D. A representative snapshot of the simulation for $N_L = 5$ is shown in the Supplemental Material

the water models. These benchmarks showed that the OPLS-AA-SPC/E combination is among the one which produces well the structural, electronic and hydration thermodynamic properties for protein-water systems [71,72]. The simulations with concentrated lecithin solution were performed using number of lecithin molecules $N_L = 5$, $N_L = 10$, $N_L = 15$ respectively. The dimensions of the simulation box were 10 nm in the cubic geometry, containing 30634 water molecules. The net charge of the protein was +2, and therefore, two negative chloride counterions were inserted to neutralize the system. Furthermore, to assess the sensitivity of different choices of the POPC molecules positions at $t = 0$ in the simulation box, three different initial conditions were generated for each concentration of the lecithin solution. A cut-off radius of 1.2 nm was used to create a non-bonded pair list. For the short-range non-bonded interactions a cut-off length at 1.1 nm was chosen for a shifted Lennard-Jones potential while the long-range electrostatic interactions were taken into account via Particle Mesh Ewald-Switch [73] (PME-switch) method with a Coulomb switching cut-off at 1.2 nm. A long-range dispersion correction was applied for truncating the van-der-Waals interactions. All bonds were constrained using the LINCS algorithm [74]. A time step of 2 fs was used for the Verlet integrator. All simulations were conducted in the canonical ensemble (NVT) at 298.5 K using the velocity-rescale thermostat [75] with a time-constant of 0.1 ps.

Before each NVT simulation, we equilibrated the density of the system within the NPT ensemble using the Parrinello-Rahman barostat [76] for 5ns. The production runs for all simulations after initial equilibration was extended up to $1.2 \mu s$. In order to assess the role of increasing concentration of lecithin, we used four different values of lecithin concentration, corresponding to $N_L = 0$ (RBD + water), 5 (RBD + lecithin + water), 10 and 15 lecithin molecules in the simulation box. For each simulation containing lecithin, we ran three inde-

pendent simulations each with different initial condition.

The binding energy of the SARS-CoV-2 receptor binding domain (RBD) and lecithin complex was calculated using the molecular mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method as implemented in Gromacs. For details of the algorithm, the reader is referred to the original work [77]. In this method the binding energy includes an enthalpic contribution coming from the sum of van-der-Waals and electrostatic interactions between the lecithin and protein. The contribution of the polar solvation energy to the free energy was determined by solving Poisson-Boltzmann (PB) equation, while the nonpolar contribution of the solvent effect was determined using the solvent accessible surface area (SASA) and effective surface tension. The linear Poisson-Boltzmann equation was used to determine the polar solvation energy contribution. In these calculations, we use a dielectric constant of 80 for the solvent, and a value of 4 for that of the solute. We used two different salt concentrations of 0M and 0.15M within the Poisson-Boltzmann theory to get some theoretical error bars on how the ionic concentration affects the polar solvation energy. For the non-polar solvation energy, a surface tension constant was set to $0.022 \text{ kJ/mol } (\text{\AA}^2)$ with a solvent probe radius of $1.4 \text{\AA}$.

4 Results

In order to build our intuition on the nature of the interactions between lecithin and the spike protein, we begin by inspecting representative snapshots of our molecular dynamics simulations (see Supplementary Movies 1, 2, and 3 for graphical representations of simulations done with lecithin concentrations $N_L = 5$, $N_L = 10$, and $N_L = 15$ respectively). Figure 2b shows the initial

condition in a zoomed-in snapshot of the simulation box for $N_L = 10$ lecithin molecules. Over time, lecithin molecules—initially at random positions in the simulation box—diffuse into the hydration shell of the protein and appear to have an affinity for certain regions of the spike protein. More specifically, we observe that lecithin molecules adhere to the RBM zone as well as Zone 2. Figures 2c–d are representative snapshots taken after $1\mu\text{s}$ simulation time for lecithin concentrations corresponding to $N_L = 10$ and $N_L = 15$ illustrating this phenomenon.

There are several interesting features that one can observe in these binding events involving lecithin. Firstly, lecithin molecules stick around the RBM and Zone 2 either as single molecules (Fig. 2c) or in the form of clusters (Fig. 2d). Furthermore, the simulations also reveal that lecithin docks to the viral protein mostly involving the hydrophobic non-polar tail. Overall, Fig. 2d shows the aggregation of lecithin into two clusters, one near the RBM and another in close proximity to Zone 2 (cf. Fig. 1b). Note that Zone 2 is exposed to the solvent in our simulations, but is bound to the core of the spike protein in the virus, hence it may be accessible to lecithin when the spike-protein trimer opens due to fluctuations or interactions with other proteins.

In the following, we provide a quantitative analysis of our simulations, by first looking in Sec. 4.1 at the global structural behavior of the SARS-CoV-2 RBD by monitoring various structural quantities such as the radius of gyration (RG) of the RBD and the root mean square fluctuations (RMSF) of all the amino acids. To investigate the protein-lecithin interactions at the atomic level, we present in Sec. 4.2 insights about the energetics of the binding events reporting estimates of the binding energetics of lecithin molecules with RBD. In Sec. 4.3, we report estimates of the entropy change and of the binding free energy of lecithin with the RBD. We then provide in Sec. 4.4 a detailed contact map analysis disentangling the interactions involving the polar and non-polar groups of the lecithin molecules with different SARS-CoV-2 RBD residues. Next, we show in Sec. 4.5 that the interaction of the hydrophobic parts of lecithin with the spike protein, reveals the crucial role of water during the interaction of the spike protein with its aqueous environment.

4.1 Structural analysis

To monitor the change in the structure of SARS-CoV-2 RBD resulting from its interaction with lecithin, we first compute the radius of gyration (RG) of the RBD and plot global distributions averaged over all three independent simulations for each lecithin concentration. In particular, we evaluate $\text{RG}(t) = (\sum_i m_i |\mathbf{r}_i(t)|^2 / \sum_i m_i)^{1/2}$, where m_i and $\mathbf{r}_i(t)$ is the mass and position of each i -th atom respectively, with respect to the center of mass of the molecule to which it belongs to.

Fig. 3 **a** Distribution of radius of gyration of SARS-CoV-2 spike protein receptor binding domain RBD at different concentrations of lecithin (see legend, where N_L denotes the number of lecithin molecules). The data is obtained from three different initial conditions for each lecithin concentration. **b** Average radius of gyration of the RBD protein including the statistical error. The trends indicate an increase in the mean RG of the RBD when increasing the lecithin concentration

The distributions of the RGs (Fig. 3a) show that lecithin significantly changes the structure of the RBD and that this effect is highly sensitive to the concentration of lecithin in water. Figure 3b illustrates the average RG computed for the RBD protein including the statistical error. The trends indicate an increase in the mean RG of the RBD when increasing the lecithin concentration.

To understand further how lecithin affects the structure and fluctuations of the RBD protein, we determined the root mean square fluctuation (RMSF) of each amino acid and examined how it changed upon the addition of lecithin. This analysis is illustrated in Fig. 4. The RMSF difference is computed for the case when protein is in solution with $N_L > 0$ to the case when $N_L = 0$. As seen from the figure by introducing higher concentrations of lecithin, the RMSF appears to increase in some regions of the protein and decrease in others. Specifically, in the RBM domain, this effect is very sensitive to the lecithin concentration.

Fig. 4 Root mean square fluctuation (RMSF) of SARS-CoV-2 RBD residues for different number of lecithin molecules in solution (see legend). For each residue, we plot the RMSF obtained in our simulations minus the value obtained for zero lecithin concentration i.e. $N_L = 0$

4.2 Lecithin-RBM binding energetics

Our preceding results point to the fact that lecithin can form favorable hydrophobic interactions with the RBM domain (see Fig. 2d) and that this binding alters the structure of the spike protein (see Fig. 3). It is beyond the scope of the current work to determine the binding free energies using the full atomistic simulations. Instead, we turned to using estimates given by the molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) as implemented in Gromacs [77] to investigate the binding energy of lecithin to the protein over the course of the microsecond simulations we performed. This approach has been successfully used in numerous other biophysical applications [78–82].

Figure 5a shows the evolution of the binding energy estimate per lecithin molecule over the course of our microsecond simulations in solution with $N_L = 15$ lecithin molecules. The three curves show that the change in interaction energy computed from the MM-PBSA at zero salt concentration, covers the range $-20k_B T$ to $-5k_B T$ per lecithin molecule as illustrated in Fig. 5b which show the average binding energy obtained as a function of the POPC concentration from these time series. We have also found that the binding energy is enhanced to the range $-25k_B T$ to $-15k_B T$ when increasing the ionic concentration to 0.15M in a mean field manner through Poisson-Boltzmann theory (see Supporting Information). This result is also consistent with the fact that we observe few detachment events of lecithin molecules from the RBD in these simulations that are triggered by $\sim k_B T$ energy fluctuations.

We would like to underscore the point that the ionic concentrations are only being included in an effective manner through the MM-PBSA and thus the changes in binding energy do not come from explicit changes induced by the ions on the structure of the protein or lecithin. The increase in the binding energy arises

Fig. 5 **a** Estimate of the binding energy (in units of $k_B T$ per lecithin molecule) between lecithin and SARS-CoV-2 RBD as a function of time, obtained by applying the MM/PBSA method to our molecular dynamics in solution with $N_L = 15$ lecithin molecules. The different lines (blue, cyan, teal) are obtained for three different initial conditions and the horizontal dashed lines are set to $\pm k_B T$. **b** Average binding energy over time and over different initial conditions as a function of the number of lecithin molecules

from the effect of screening when solving the Poisson-Boltzmann equation. Larger salt concentration implies reduced screening length so that at the same distance, the electrostatic potential is lower. The methodology we are using is also employed in previous studies looking at the energetics of binding of natural products and other candidate drug molecules as inhibitors to SARS-CoV-2 [83–86]. Interestingly, our estimates (Fig. 5b) are comparable to the binding affinity of different drug molecules with the SARS-CoV-2 RBD trimer ranging from -5kcal/mol to -12kcal/mol [87]. A recent thorough study using molecular docking identified approximately 100 natural product inhibitors with binding energies of about -9kcal/mol to the TMPRSS2 protease [28].

In summary, the binding energetics based on enthalpic contributions of lecithin to the RBD observed in our simulations suggests that phospholipids may play an important role in the interactions involving the RBD under cellular conditions. The binding energies we report in Fig. 5 do not include entropic contributions

Fig. 6 Conformational entropy change of the lecithin molecules as a function of time. The colored curves report the change observed in the three initial conditions (IC1, IC2, IC3) simulated for each concentration of POPC molecules: **a** $N_L = 5$, **b** $N_L = 10$, and **c** $N_L = 15$. We estimate the

entropy using the MIST₂ approach with a moving time window of 0.1 ns, assuming that the entropy does not relax appreciably within this period. The gray curves (MA) averages the three initial conditions over a narrower window of 0.05 ns. Error bars are given by one standard deviation

which ultimately determine the free energy of binding. In order to assess the role of these effects, in the next section we examine the change in entropy of the lecithin molecules.

4.3 Lecithin entropic contributions

The binding of the POPC molecules to the protein will obviously lead to a reduction in their conformational entropy. In order to assess the magnitude of this entropic change, we used an information-theoretical technique namely, the so-called Maximum Information Spanning Trees (MIST) [88]. In this approach, the entropy of a molecule is evaluated as a truncated expansion in contributions, involving mutual information between an increasing number of the molecule's degrees of freedom. Such an expansion is constructed so as to provide the tightest upper bound for the chosen order of approximation. Because the MIST provides a direct estimate of the entropy, assumptions often invoked in, for example, the quasi-harmonic approach are thus avoided [89–91].

There exist an efficient graph-theoretical algorithm for evaluating the second-order expansion MIST₂ [88, 92, 93], which we adopt in the following. Two implementations of this approach have been described e.g. in Refs. [92, 93], which differ by the coordinate transformations used to remove the translations and rotations of a molecule as a whole. Instead, we developed and implemented our custom implementation of the MIST₂ algorithm which removes molecular translations and rotations by using a Cartesian reference frame aligned with the principal axes of the molecule's tensor of inertia, with the origin placed at the molecule's center of mass. Our algorithm is based on the MDAnalysis package [94, 95] and uses the Kozachenko-Leonenko nearest-neighbour estimate of entropy and mutual information [96].

Figure 6 shows how the entropy changes over time for the various concentrations of POPC. The entropy at each time point is computed by averaging over a moving time window of 0.1 ns for each initial condition, whereas the gray curves average over all the initial conditions using a narrower window of 0.05 ns. The entropic contri-

bution of the overall free-energy change ranges between -15 to $0 k_B T$ and is rather sensitive to the concentration of POPC molecules in the solution (see Table 1). The negative values of the entropy difference imply that the reduction of conformational freedom does not favor binding of lecithin with the RBD domain. At the lower concentration $N_L = 5$ the entropic contribution is $-15 k_B T$, whereas for $N_L = 15$ it is essentially zero within the statistical error. These results suggest that at the lower concentrations of lecithin, there is no substantial driving force for binding, as the competing enthalpic and entropic effects are similar. At the higher concentrations of POPC molecules, the enthalpy gain overwhelms the small penalty of the entropy loss. Table 1 summarises these results showing how the binding-free energy estimate changes as a function of the concentration of POPC.

4.4 Interatomic contacts between lecithin and SARS-CoV-2 RBD

In this section, we investigate the interatomic contacts that form between lecithin and spike protein. To this aim, we plot in Fig. 7 contact maps between all the heavy atoms¹ in the protein residues of SARS-CoV-2 RBD and lecithin molecules for the lecithin concentration value $N_L = 15$. We focus this analysis on two quantities, namely the average interatomic distance (Fig. 7a, b) and the average interaction time (Fig. 7c, d) between lecithin and the different viral protein zones (see Fig. 1). Figure 7a and c show the interaction distances and times that lecithin forms with the entire RBD. Consistent with Fig. 2, we observe that there are some hot-spot regions where lecithin docks preferentially, in particular, Zone2 and RBM. Within these regions, the interatomic distances between lecithin and the viral protein are less than one nanometer. Since the RBM zone is the one that interacts with ACE2 we focus on examining its contacts with lecithin in Fig. 7b. Specifically, lecithin appears to form close contacts with

¹ All our contact maps are evaluated taking into account the positions of all except the hydrogen atoms, i.e. only the heavy atoms.

Table 1 Binding free-energy contributions of lecithin with SARS-CoV-2 RBD (per lecithin molecule) as a function of the number of POPC molecules in the solution (N_L): free-energy change ($\Delta F = \Delta E - T\Delta S$), and the energetic (ΔE) and entropic ($T\Delta S$) contributions

N_L	ΔE ($k_B T$)	ΔS (k_B)	ΔF ($k_B T$)
5	-21 ± 4	-15 ± 2	-6 ± 6
10	-15 ± 3	-6 ± 2	-9 ± 5
15	-11 ± 4	-0.7 ± 0.9	-10 ± 5

The free energy-change contributions are obtained by computing the difference between their final value (1 μ s after equilibration) and their value right after equilibration, and averaging over the three initial conditions used for each value of lecithin concentration

Fig. 7 Contact maps for interactions between different zones of SARS-CoV-2 receptor binding domain (RBD) and $N_L = 15$ lecithin molecules (L). **a** Contact map of distances for the whole RBD. **b** Zoomed-in view of **a** for the receptor-binding motif (RBM). **c** Contact map of interaction times for the whole RBD, at distances below 1 nm. **d** Zoomed-in view of **c** for the receptor-binding motif (RBM). In **c–d** the time is expressed in % relative to the total simulation time

amino acids 473–490, which contain ACE2-interacting residues (cf. Fig. 1c).

Figure 7c, d show contact maps of the interaction times associated with lecithin and the RBD. In order to quantify interaction times, we count the time elapsed within a threshold distance of 1 nm corresponding to the typical range of van-der-Waals forces. We find that the average interaction time between lecithin and the RBD is on the order of hundreds of nanoseconds. As expected, the core of the protein (Zone 3) is characterized by the weakest binding. We observe similar trends in our simulations at lower concentrations of lecithin, i.e. for $N_L = 5$ and $N_L = 10$ lecithin molecules (see Supporting Information).

To gain further insights into the physical driving force between the molecular interactions, we investigate the average contact maps of polar and non polar

Fig. 8 Average contact maps between different zones of SARS-CoV-2 receptor binding domain (RBD) and lecithin polar **a** and non polar **b** groups, see text for more details. The bottom bars are obtained by zooming-in the lecithin-protein contacts in panel A (left bottom bar) and B (right bottom bar)

groups of lecithin molecules with the residues of the RBM. We evaluate the average interatomic distance between the RBM atoms and two different parts of lecithin molecules: (i) the hydrophilic polar group (see Fig. 8a); and (ii) the hydrophobic nonpolar chain (see Fig. 8b). Notably, the interatomic distance between all the subdomains of the RBM and the hydrophobic tail of lecithin molecules is significantly smaller than the distance to the hydrophilic head. Therefore the lecithin-RBM contacts are mainly dominated by hydrophobic interactions and in fact, this is enhanced under higher concentrations of lecithin.

4.5 Dewetting of the receptor-binding motif

Water plays an instrumental role in tuning the structural and dynamical properties of biological systems [97]. In the context of our work, numerous studies emphasized depletion of the hydration shell as an important factor facilitating the hydrophobic interactions between proteins [98,99]. The preceding analysis shows that hydrophobic interactions between the non-polar groups of lecithin form close contacts with the RBM.

To find a further evidence of the hydrophobic interactions revealed in the previous analysis, we calculated the radial distribution of water molecules $g(r)$ for six-

Fig. 9 Dewetting of the RBM domain in the lecithin solution. We analyze radial distribution functions $g(r)$ of water molecules as a function of distance r from two (Ala475 and Phe486) of the 16 amino acids of the RBM domain which are involved in binding with ACE2 (highlighted as red dots in Fig. 1c). This analysis was performed on two 1.1 μ s-long trajectories—with a high number (a–c) and a low number (b–c) of binding events between the lecithin molecules and the RBM domain respectively—after discarding 900ns for the transients decay in the system with $N_L = 15$ and using as reference the full 1.2- μ s-long trajectory with $N_L = 0$. **a** A representative snapshot of a simulation with many binding events between the solute and the RBM (red ribbon)—five lecithin molecules docking to the protein domain. **b** Radial distribution function for the same simulation (solid lines) and for the reference simulation with $N_L = 0$ (dashed lines);

the arrow indicates the increasing trend of the hydration shell's outer radius r_o defined as $g(r_o) = 0.5$. **c** The percentage changes of r_o , with respect to the reference $N_L = 0$, in the simulation with the high number of binding events for all the sixteen residues that we examined; Ala475 and Phe486 are singled out by the same colors as in the panel B. A high number of the binding events **a** is accompanied by a striking increase of the hydration-shell radius near these two residues. **d** A representative snapshot of a simulation with low number of binding events—one lecithin molecule docking to the RBM domain. **e** Radial distribution function for the respective simulation (solid lines) shows a weak increase of the hydration-shell radius in comparison with the reference case $N_L = 0$. **f** The percentage changes of r_o in the simulation with the low number of binding events for all the sixteen residues

teen residues highlighted as “RBM hotspots” with red circles in Fig. 1c. These hotspots have been implicated in the docking mechanism of SARS-CoV-2 and ACE2 [100].

As shown in Fig. 9a, lecithin molecules deplete the hydration shell of the RBM, whose outer radius r_o thus increases. This dewetting effect is most pronounced near two hydrophobic residues—alanine Ala475 and phenylalanine Phe486. The relative change of the hydration-shell radius near these residues exceeds 100% (see Fig. 9a). Low number of the lecithin molecules docking to the RBM may be observed also at higher concentrations of the solute (Fig. 9d). As expected, the dehydration effect observed in this case is less pro-

nounced. Nonetheless the number of binding events between lecithin and RBM tends to increase together with the solute's concentration.

The changes in the solvation structure around the RBM domain triggered by the binding of lecithin creates a large excluded volume occupied by lecithin molecules that prevents water from aggregating close to the receptor binding motif. On the other hand, the preceding analysis shows that there is quite a variety of solvent accessibility across the RBM binding. This suggests that tuning the chemical properties of the surfactant, for example using an ionic molecule, may lead to a more pronounced role of the solvent fluctuations.

5 Discussion

In this work we have performed all-atoms molecular dynamics simulations of the interaction between SARS-CoV-2 receptor binding domain (RBD) and phospholipid molecules (lecithin POPC) diffusing in water. Our main results are summarized as follows: (i) lecithin induces a conformational change in the RBD and its receptor binding motif which is revealed by an increase of the radius of gyration with the lecithin concentration; (ii) lecithin molecules bind mostly by docking their hydrophobic tails into the receptor-binding motif (RBM) domain located within the RBD. This hydrophobic interaction occurs at distances that are smaller than 1 nm and with significant binding free energies for large values of the POPC concentration (see Table 1). (iii) Hydrophobic interactions play a key role in the lecithin-RBD binding, as this is accompanied by a dewetting of water molecules in the receptor binding motif. Taken all together, these results provide insights about the possible role of surfactant molecules such as phospholipids present in liposomal nasal sprays in mediating the interactions of SARS-CoV-2. It will be thus interesting in the future to extend our analysis to the case of POPC micellar aggregations, as those present in liposomal nasal sprays.

Phospholipids may be capable of preventing infection to a certain extent and may be used in combination with other therapies. Note that this idea is not unwarranted as phospholipids have previously been employed to treat coronavirus [29]. On the other hand, phospholipids have also been successfully applied to reduce the viral activity of RSVs [30,31]. The approach has been subject of numerous examinations in the recent years, especially in-vitro studies, suggesting e.g. a significant potential for short-term prevention and therapeutic application for respiratory virus infection [33]. Moreover, the role of the pulmonary surfactant has been highlighted and proposed as a potential treatment approach even for severe cases of COVID-19 [101–105]. All in all, the manner in which surfactants are able to alter and mediate the interactions of viral proteins in the cell offers many interesting and promising possibilities and improve our understanding of the interaction between phospholipids and receptor-binding motif of SARS-CoV-2 at the nanoscale. It remains to be investigated in controlled clinical studies whether lecithin-based therapies may contribute to improve therapeutic measures, reduce infections and improve containment of the enduring COVID-19 pandemic. Another area that deserves attention are the role of mutants [106,107] in how they may change the binding affinity of putative blockers of SARS-CoV-2 RBD. The mechanisms of the efficiency of POPC action on mutants is beyond the scope of this paper, yet we can only speculate that mutants that enhance the exposure of

hydrophobic groups in the RBD domain may enhance the binding of POPC.

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Author contribution statement

MNQ performed all the molecular dynamics simulations. All authors analyzed data. IG provided computing support. All authors wrote the manuscript. OG, AH, and ER proposed and directed the project.

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