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EML webinar overview: Simulation-assisted discovery of membrane targeting nanomedicine

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ABSTRACT

The COVID-19 pandemic has brought infectious diseases again to the forefront of global public health concerns. In this EML webinar (Gao, 2020), we discuss some recent work on simulation-assisted discovery of membrane targeting nanomedicine to counter increasing antimicrobial resistance and potential application of similar ideas to the current pandemic. A recent report led by the world health organization (WHO) warned that 10 million people worldwide could die of bacterial infections each year by 2050. To avert the crisis, membrane targeting antibiotics are drawing increasing attention due to their intrinsic advantage of low resistance development. In collaboration with a number of experimental groups, we show examples of simulation-assisted discovery of molecular agents capable of selectively penetrating and aggregating in bacterial lipid membranes, causing membrane permeability/rupture. Through systematic all-atom molecular dynamics simulations and free energy analysis, we demonstrate that the membrane activity of the molecular agents correlates with their ability to enter, perturb and permeabilize the lipid bilayers. Further study on different cell membranes demonstrates that the selectivity results from the presence of cholesterol in mammalian but not in bacterial membranes, as the cholesterol can condense the hydrophobic region of membrane, preventing the penetration of the molecular agents. Following the molecular penetration, we establish a continuum theory and derive the energetic driving force for the domain aggregation and pore formation on lipid membrane. We show that the energy barrier to membrane pore formation can be significantly lowered through molecular aggregation on a large domain with intrinsic curvature and a sharp interface. The theory is consistent with experimental observations and validated with coarse-grained molecular dynamics simulations of molecular domain aggregation leading to pore formation in a lipid membrane. The mechanistic modelling and simulation provide some fundamental principles on how molecular antimicrobials interact with bacterial membranes and damage them through domain aggregation and pore formation. For treating viral infections and cancer therapy, we discuss potential size- and lipid-type-based selectivity principles for developing membrane active nanomedicine. These studies suggest a general simulation-assisted platform to accelerate discovery and innovation in nanomedicine against infectious diseases.

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1. Introduction

One of the most important public health issues that the world faces today is the threat of global infectious disease outbreaks. In the last several decades, newly identified and re-emerging virus related infectious diseases including human influenza, HIV/AIDS, severe acute respiratory syndrome (SARS), dengue, malaria, Ebola, and Middle East respiratory syndrome (MERS) have demonstrated serious epidemic potential with devastating loss of life and wealth. The COVID-19 pandemic has brought infectious diseases again to the forefront of global public health concerns. As of Jun. 4, 2020, more than 6 million people had been infected and hundreds of thousands had died worldwide [1]. In addition to the virus, bacterium is another major human pathogen that are responsible for a wide range of life-threatening infections [2]. Antibiotics are widely prescribed and can effectively kill or inhibit the growth of metabolically active bacteria [3,4]. However, in recent years, the increasing prevalence of antibiotic resistant strains and the
ability of bacterial cells to convert to a quiescent subpopulation (often referred to as “persisters” [5]) that exhibits a high level of tolerance to most antibiotics [6–10] have caused clinical challenges that may eventually lead to a crisis level shortage of effective antibiotics for treating bacterial infections. A recent report led by the world health organization (WHO) warned that 10 million people worldwide could die of bacterial infections each year by 2050 [11]. Consequently, there is an urgent societal need to develop novel antibiotics with unconventional antimicrobial strategies.

Antimicrobial chemotherapy seeks to eradicate the infecting pathogen from its host in the shortest possible treatment period. Most conventional antibiotics targeting biosynthetic processes that occur in actively growing bacteria, including the biosynthesis of proteins, RNA, DNA, peptidoglycan and folic acid [12], fail to meet the challenges related to the mutation, resistance and persisters [6–10]. On the other hand, the bacterial membrane, as an essential structural and functional component of biological organisms, providing selective permeability for cellular homeostasis and metabolic energy transduction even if the bacteria are in the dormant state, has attracted increasing attention as targets for new generation antibiotics, as membrane-active agents are inherently effective on sleeping bacterial persisters and also difficult for the bacterial to acquire resistance [13]. Viruses, especially the RNA viruses such as the influenza and coronaviruses, are highly susceptible to mutations [14]. Since the outbreak of SARS-CoV-2 in January, abundant mutations have been reported in just a few months [15–18], and membrane active nanomedicine might be a promising but underexploited approach to effectively counter viral mutations.

In this webinar, we discuss some examples of simulation-assisted discovery of molecular agents which are promising candidates for a new generation of membrane-active antibiotics and potential application of similar ideas to the current pandemic. As shown in Fig. 1, the webinar covered the antimicrobial mechanisms of synthetic antibiotics in attachment, penetration and aggregation in the cell membrane, leading to membrane distortion, pore formation and rupture. Some derived theory and physical insights can also be applied to some antiviral agents that seem effective for a broad range of viruses by disrupting the lipid envelop [19], which may help discover, design and fabricate membrane targeting antiviral agents to combat COVID-19. The study suggested a simulation-assisted platform to accelerate discovery and innovation in nanomedicine.

### 2. Membrane targeting antibiotics

*Staphylococcus aureus* is an opportunistic human pathogen carried by approximately one third of human populations, and infections by *S. aureus* remain a major cause of death [20]. To demonstrate how simulation can assist the discovery of membrane-active antibiotics, we started from all-atom molecular dynamics (MD) simulations of the interactions between a class of retinoids (Fig. 2a) and the lipid bilayer membrane of *S. aureus*. A previously established lipid bilayer composed of DOPC/DOPG at a 7:3 ratio was adopted to model the negatively charged *S. aureus* membrane [21]. Four synthetic retinoids (Fig. 2a) were systematically investigated, among which CD437 and CD1530 were identified as potential antibiotics through a biological screening assay [22], while adarotene and adapalene were selected because of their similar structures as CD437 and CD1530. The simulations showed that the carboxylic acid and the phenolic groups of CD437, CD1530 and adarotene help anchor these retinoids to the surface of the membrane bilayer through binding with hydrophilic lipid heads, followed by penetration into the bilayer and becoming embedded in the outer membrane leaflet, inducing substantial perturbations to the membrane. In comparison, adapalene, a molecule of similar structure, cannot penetrate into the bilayer as the hydrophobic methoxy group does not bind to the lipid heads (Fig. 2b). Free energy profiles associated with the membrane penetration of these molecular agents were calculated based on steered molecular dynamics and umbrella sampling [23–25]. The free energy mapping shows that membrane penetration by CD437, CD1530 and adapalene is energetically favourable with barriers on the order of thermal fluctuation while adapalene exhibits a high energy barrier and an unfavourable transfer energy against membrane penetration (Fig. 2c). In comparison with CD437 and CD1530, adarotene shows higher energy barrier and smaller transfer energy, hence less favourable for membrane penetration. These results are consistent with the biomembrane-mimicking giant unilamellar vesicles (GUVs) experiments (Fig. 2d), where domain aggregation and pore development are observed on the surfaces of the GUVs exposed to CD437, CD1537 and adarotene, while the GUVs exposed to adapalene remain intact. Further *in vivo* and *in vitro* experiments indicated that CD437 and CD1530 are potential new membrane-active antibiotics with fast bacteria killing rate and low resistant development, while adarotene and adapalene exhibit lower and no antimicrobial activities, respectively [26].

The discovery of this class of retinoids as new antibiotics demonstrated the powerful roles of molecular simulations. The simulations and free energy mapping revealed atomistic insights on how molecular agents attach on and penetrate into the bacterial membranes, and the excellent agreement with experiments.
indicated that modelling and simulation can effectively capture whether a given compound is a potential candidate for membrane-active antibiotics.

3. Cholesterol-based membrane selectivity

One of the major challenges in developing membrane targeting agents is to ensure their selectivity for pathogen since those of low membrane selectivity are typically toxic to mammals [5]. Despite many common features among all biological membranes, the lipid compositions of bacterial and mammalian cell membranes are quite different. Specifically, unlike the negatively charged S. aureus membrane, the outer leaflet of mammalian cell membrane is neutral, with cholesterol ranging from 20 to 50 mol% [27–29]. This intrinsic difference between bacterial and mammalian cell membranes provides a basis for developing membrane targeting antibiotics.

Taking a clinically approved anthelmintic drug bithionol (Fig. 3a) as an example, we found that the simulations can also help capture membrane selectivity. All-atom MD simulations show that bithionol can be recruited to the bacterial membrane surface, penetrates into the membrane and embeds in the outer leaflet of the lipid bilayer. In contrast, bithionol fails to penetrate mammalian mimetic lipid bilayers with 7:3 POPC/cholesterol ratio [27,28] (Fig. 3b). The free energy mapping confirms that penetration of bithionol into the mammalian mimetic membrane is energetically unfavourable (Fig. 3c). Moreover, the presence of cholesterol leads to a more ordered alignment of the membrane lipids, condenses the hydrophobic region of the membrane and decreases membrane fluidity. As a result, the energy barrier and transfer energy associated with membrane penetration increases monotonically with the percentages of cholesterol, suggesting that cholesterol plays a key role in membrane selectivity [30].

The GUVs experiments on the effects of bithionol on different lipid bilayers are in agreement with the simulations: bacterial mimetic GUVs exposed to biothionol undergo domain aggregation, pore development and rupture, while no such effects are observed on mammalian mimetic GUVs. Cell-based assays further demonstrate that bithionol indeed exhibits bactericidal activity and low levels of toxicity to mammalian cells [30].

Our modelling and simulation demonstrated that the selectivity of bithionol for bacterial membranes correlates with its ability to penetrate and embed in bacterial-mimic lipid bilayers, but not in cholesterol-rich mammalian-mimic lipid bilayers. This then provided a fundamental insight into the molecular mechanisms by which membrane-active molecular agents selectively disrupt bacterial over mammalian membranes.

4. Domain aggregation and pore growth in lipid membrane

The above studies demonstrated that modelling and simulation can predict the ability of molecular agents to selectively penetrate into lipid membranes of pathogen, providing a theoretical basis to understand membrane activity and selectivity of membrane active nanomedicine. However, we also found that, while some molecular agents (such as PQ401) can penetrate into the mammalian-mimetic membrane, they do not disrupt mammalian-mimetic GUVs and red blood cells [31]. This observation suggests that penetration is only the first and necessary step, but not sufficient for the final rupture of the lipid membrane.
More detailed experimental observations on the evolutions of GUVs exposed to membrane-active compounds indicate that the entire interaction process leading to the final rupture of lipid membranes include further intricately coupled physical processes such as diffusion, domain aggregation, deformation, pore formation and growth (Figs. 1, 2d, 3d), which raise a number of open questions. For example, what is the driving mechanism for domain aggregation? Why is there a characteristic domain size? Why does domain aggregation facilitate pore formation? What are the effects of homogeneous vs. interfacial nucleation of pores? How does the spontaneous curvature of domain affect pore formation? How does the vesicle size affect pore formation? How to predict the energy barrier and time scales for pore formation? In this webinar, we highlighted some selected questions related to the characteristic domain size and pore formation and growth.

Regarding domain aggregation, consider an inclusion domain \( \Omega_2 \) of inserted molecular agents with area \( A_2 \) in an infinite membrane \( \Omega_1 \). The domain adopts an axisymmetric shape while inducing an intrinsic curvature \( \kappa_2 \). The membrane has bending modulus \( \kappa_2 \) and is under remote tension \( \Sigma \). When the aggregated domain incorporates dispersed molecules, a mixing energy per unit area \( \Delta G \) is induced, which includes the interaction energy between the dispersed molecules and lipids as well as the entropy change during the aggregation process. For simplicity we ignore the line tension on the domain interface. We find that if \( -\kappa_2 C_2^2/2 < \Delta G < 0 \), the domain has an optimal size. Specifically, when \( -\kappa_2 C_2^2/2 \ll \Delta G < 0 \), the domain size can be written as \( A^*_2 \approx -\frac{\kappa_2}{\kappa_2} \). Thus, we conclude that a negative mixing energy drives domain growth while the intrinsic curvature and surface tension limit its size.

We also modelled the pore (denoted by \( P \)) growth in the presence of an inclusion domain. Apart from the bending energy \( \gamma_1 \) and \( \psi_1 \) in the inclusion and the lipid membrane, the system energy also includes three line tensions, namely \( \gamma_{1-P} \) on the lipid–inclusion interface, \( \gamma_{1-P} \) on the lipid–pore interface and \( \gamma_{1-P} \) on the inclusion–pore interface. We find that both the bending energy and the lipid–inclusion line tension contribute a negative energy variation during the pore growth process, thereby facilitating the pore growth. Following this discovery, it is also shown that smaller vesicles have lower energy barrier for rupture since their high curvature leads to higher bending energy, a conclusion in line with the experimental observations [31]. Such size effect can potentially be utilized to understand the recently developed antiviral agent by Cho et al. [19] with a strong size-based selectivity. They showed experimentally that this antiviral agent can selectively rupture smaller liposomes and have a broad-spectrum efficacy against a wide range of viruses. Given that SARS-CoV-2 possesses a similar size, this antiviral agent may also be a potential candidate for treating COVID-19. We also proved the influence of domain size on pore formation. With numerical solutions, we find that larger domains exhibit lower energy barrier for pore growth than smaller ones. As the interfacial pore grows, the domain shape remains almost unperturbed in large domains. Yet in smaller domains, either the shape of the pore or the domain can be distorted with substantial increase in the energy cost.

5. Conclusions

In this webinar, we summarized some recent studies with a long term goal to establish a simulation-assisted discovery platform for membrane active nanomedicine capable of selectively penetrating and aggregating in bacterial/viral lipid membranes, causing membrane permeability/rupture. Our multiscale modelling and simulation studies demonstrated that the membrane activity of molecular agents correlates with their ability to enter, perturb and permeabilize the lipid bilayers. Further study on different cell membranes revealed the molecular mechanism of cholesterol-based selectivity. Following the molecular penetration, we established a continuum theory and derived the energetic driving force for domain aggregation and pore growth on lipid membranes. We showed that there exists an optimal domain size and that the energy barrier to membrane pore formation can be significantly lowered through molecular aggregation on a large domain with intrinsic curvature and a sharp interface. The curvature effect of lipid membrane derived from the theory may have important implications to develop membrane targeting antiviral agents with size-based selectivity.

The current drug discovery process for antibiotics using biological screening methods (such as \( C. elegans \) survival [22]) are usually expensive, time consuming and inefficient. Moreover, it has been difficult to identify the underlying mechanisms of positive hits in the screening and improve them subsequently. Here, we showed that mechanistic modelling and simulations can help reveal some fundamental principles on how molecular antimicrobials interact with bacterial membranes and damage them through domain aggregation and pore formation. Our study highlights a promising approach to establish a simulation-assisted platform to accelerate the screening, assessment, design and optimization of membrane targeting nanomedicine at reduced cost against infectious diseases and, more importantly, to effectively counter increasing challenges with pathogen resistance/mutation.

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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