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Modeling selectivity of antimicrobial peptides: how it depends on the presence of host cells and cell density

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Antimicrobial peptides (AMPs), naturally-occurring peptide antibiotics, are known to attack bacteria selectively over the host cells. The emergence of drug-resistant bacteria has spurred much effort in utilizing optimized (more selective) AMPs as new peptide antibiotics. Cell selectivity of these peptides depends on various factors or parameters such as their binding affinity for cell membranes, peptide trapping in cells, peptide coverages on cell membranes required for membrane rupture, and cell densities. In this work, using a biophysical model of peptide selectivity, we show this dependence quantitatively especially for a mixture of bacteria and host cells. The model suggests a rather nontrivial dependence of the selectivity on the presence of host cells, cell density, and peptide trapping. In a typical biological setting, peptide trapping works in favor of host cells; the selectivity increases with increasing host-cell density but decreases with bacterial cell density. Because of the cell-density dependence of peptide activity, the selectivity can be overestimated by two or three orders of magnitude. The model also clarifies how the cell selectivity of AMPs differs from their membrane selectivity.

1. Introduction

Antimicrobial peptides (AMPs) are naturally-occurring peptide antibiotics used in the host defense of living organisms (*e.g.*, animals, plants, …).[1,2] They are relatively short, typically consisting of 20–50 amino acids. In the bulk, they often resemble random coils, but when inserted in membranes, they assume compact, amphiphilic structures (*e.g.*, α helices), as required for their antimicrobial activity (*e.g.*, membrane perturbation). AMPs are mostly cationic and thus utilize the unique 'design feature' of microbial membranes,[1] enriched with anionic lipids.[1–3] Cationic AMPs preferentially attach to and rupture microbial membranes over host cell membranes; in the latter case, anionic lipids are segregated to their inner layer (see Fig. 1). Once they gain entry into the cytoplasm, they can target key intra-cellular components (*e.g.*, DNA and proteins), leading to intra-cellular killing of microbes.[1,2]

There has been much interest in developing enhanced AMPs as potent peptide antibiotics, especially for fighting drug-resistant bacteria.[1–5] Membrane-targeting AMPs are advantageous.[1–4,6] They act *via* physical mechanisms such as pore formation[1–4,6] or anionic-lipid clustering[3] in membranes, which bacteria cannot easily avoid. In addition to rupturing bacterial membranes, they act as metabolic inhibitors[1,2] and/or immunomodulators.[7] Even though pathogens can, in principle, evolve antimicrobial resistance,[8,9] the therapeutic potential of these multitasking molecules deserves much consideration.[4,5]

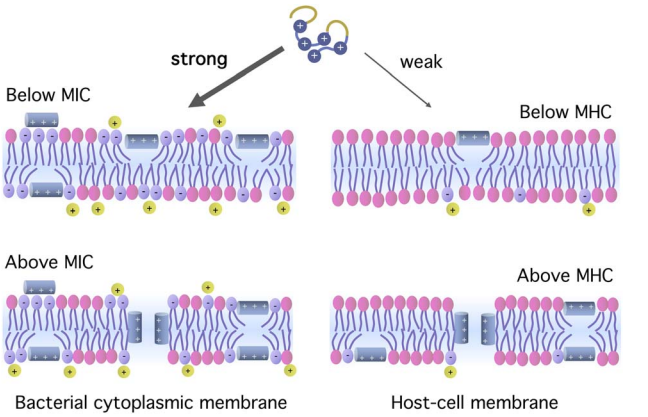

Fig. 1 Origin of peptide selectivity. Cationic antimicrobial peptides interact more strongly with bacterial membranes enriched with anionic lipids. Bound peptides can form pores in the membrane when the bulk concentration is at or above the MIC or MHC. In the figure, the membranes are only schematically illustrated, leaving out such details as membrane proteins and the presence of cholesterol in the host-cell membrane. The figure is inspired by ref. 1, 2 and 10.

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Cationic AMPs can single out bacteria through their stronger binding affinity for bacterial membranes.[1–4,6] The resulting selectivity can be quantified by the ratio of two concentrations: the minimum hemolytic concentration (MHC) and the minimum inhibitory concentration (MIC).[10,11] At or beyond this concentration, peptides can form pores in their binding membranes, as illustrated in Fig. 1. The larger the ratio MHC/MIC is for a given peptide, the more selective the peptide is. In a sizeable range of peptide concentration (~μM) between MIC and MHC, the peptide is active against bacteria while leaving the host cells unharmed.

The selectivity of AMPs is influenced by a number of factors or parameters such as their binding affinity for cell membranes, peptide trapping in (dead) cells,[12–14] cell density,[11–18] and a peptide coverage on cell membranes required for membrane rupture.[19–21] Let P/L denote the molar ratio of bound peptides to lipids. At the MIC or MHC, P/L reaches a threshold value, P/L^*. The value of P/L^* depends on the type of peptide and lipid[19–21] and is typically larger for membranes containing lipids with smaller headgroups such as phosphatidylethanolamine (PE) as in bacterial membranes. Recent studies suggest that at P/L^*, each cell consumes a certain number of peptides with some of them trapped in the cell.[12–14] This implies that the MIC or the MHC increases with increasing cell density; as a result, the ratio MHC/MIC is cell-density dependent.[11–13,16–18] The cell-density dependence is often referred to as an inoculum effect[12–15] and is known to enhance population survivability.[14]

A natural consequence of the cell-density dependence of peptide activity and selectivity is that the selectivity depends on the way it is measured.[16–18] For instance, it can be obtained by combining MIC and MHC measured separately from bacteria-only and host-cell-only solutions, respectively. In this work, the resulting selectivity is referred to as "noncompetitive" selectivity. More realistically, it can be measured from a mixture of both types of cells: "competitive" selectivity. In this case, the presence of host cells raises the MIC and influences the ratio MHC/MIC.[13,16,22] These two approaches generally lead to different levels of selectivity. This implies that the selectivity reflects the biological setting of infected sites (*e.g.*, the degree of infection, …).

According to what is discussed above, peptide selectivity not only reflects peptide's intrinsic properties such as peptide charge and hydrophobicity, but it also depends on external parameters such as cell density and the presence of host cells. Does this mean that the selectivity should be measured for a wide range of cell density and various combinations of host cell and bacterial cell density? Recent modeling efforts, however, suggest that these two aspects (intrinsic and extrinsic) are well separated.[16,18] With an appropriate model, one can figure out the selectivity with varying cell density, once it is known at a low cell-density limit or at conveniently-chosen density. Furthermore, in the past, model lipid membranes, mimicking cell membranes, were often used for peptide activity or selectivity experiments.[10,19–21] How does the resulting membrane selectivity differ from cell selectivity measured for cells (bacteria *versus* host cells)? Peptide trapping is one of the determining factors in the latter[12–14,16] but is expected to be insignificant in the former.

Recently, we examined theoretically peptide selectivity and clarified the effects of peptide trapping on the selectivity, MHC/MIC.[16] This effort is relevant in the presence of an excess amount of host cells or for a homogeneous solution of either bacteria or host cells. Here, we extend this effort and offer a more complete picture of the activity and selectivity of AMPs, which can be used to interpret selectivity measurements or to assist with our endeavor in finding optimized peptides.

This work builds on earlier studies.[12–16] The results reported in this work, which are relevant for melittin-like peptides, suggest a rather nontrivial dependence of the selectivity on the presence of host cells, peptide trapping, and cell density. Peptide trapping can enhance or reduce the selectivity depending on how cell (host and bacterial) density is chosen. In most cases, it works in favor of the host cells, enhancing the selectivity. The presence of an excess amount of host cells (5×10^9 cells per mL) as in whole blood can raise the MIC more than 10-fold, proportionally with the density of bacterial cells. The resulting MIC still falls in a low-μM range as long as the bacterial cell density is somewhat smaller than 5×10^7 cells per mL.

Let C_B and C_H be the density of bacteria and the density of host cells, respectively, and N_p the number of peptides trapped per cell. As we raise C_B and C_H coherently so that $C_B = C_H$, the selectivity decreases in both noncompetitive and competitive cases. Similarly, in the presence of an excess amount of host cells, the selectivity decreases with increasing C_B in both cases, more so for larger N_p. In contrast, when the bacterial cell density is fixed at $C_B = 5 \times 10^4$ cells per mL or $C_B = 10^8$ cells per mL, the selectivity increases with increasing C_H, more rapidly for larger N_p. Compared to the competitive one, the noncompetitive selectivity can be overestimated by more than two orders of magnitude, depending on how C_B and C_H are chosen (see refs. 11 and 16 for related discussions).

We also clarify how the cell selectivity of AMPs differs from their membrane selectivity. While the selectivity based on model membranes is typically larger than the corresponding cell selectivity, the (relative) difference between competitive and noncompetitive selectivity is generally larger in the latter. Except for some differences, membrane selectivity and cell selectivity of AMPs are qualitatively similar to each other. If interpreted with care, the former can provide useful information about the latter.

In this work, we will focus our effort on presenting a selectivity model in a pedagogical but yet systematic manner. In our consideration, one of the main differences between model membranes and cells comes from peptide trapping in the latter. Nevertheless, we will use membrane density and cell density interchangeably; also MICs and MHCs refer to peptide concentration beyond which membranes are ruptured, whether they are model membranes or cell membranes.

This paper is organized as follows: in Section 2, we present a simple picture of how the activity and selectivity of AMPs vary with cell density for a noncompetitive and competitive medium. Section 3 introduces a Langmuir model of peptide binding.

Table 1 Definitions of symbols and acronyms

Symbol or acronym	Definition
A_{cell}	Cell surface area
A_B (A_H)	Bacterial (host) cell surface area
a_l	Lipid headgroup area
a_B (a_H)	Lipid headgroup area of bacterial (host-cell) membranes
A_p	Cross-sectional area of a peptide on the membrane surface
C_{cell}	Concentration of cells
C_H (C_B)	Concentration of host (bacterial) cells
C_p	Total peptide concentration
C_p^*	C_p at which $P/L = P/L^*$
P/L	Molar ratio of bound peptides to lipids
P/L^*	Threshold value of P/L required for membrane rupture, corresponding to MIC or MHC
MIC	Minimum inhibitory concentration
MHC	Minimum hemolytic concentration
MIC_0 (MHC_0)	MIC (or MHC) in the low-cell density limit: $C_{cell} \to 0$
μ_{bound}	Chemical potential of bound peptides
μ_{free}	Chemical potential of free peptides
N_p	Number of trapped peptides per cell
N_{pB} (N_{pH})	Number of trapped peptides per bacterial (host) cell
N_p^*	Number of trapped peptides per cell at P/L^*
N_{pB}^*(N_{pH}^*)	Number of trapped peptides per bacterial (host) cell at P/L^*
n_p	Number density of trapped peptides in a cell
n_{pB} (n_{pB})	Number density of trapped peptides in a bacterial (host) cell
N_{cell}	Number of cells
σ_p	Planar density of adsorbed peptides to membranes
k_B	Boltzmann constant
T	Temperature
V	Volume of the system
v_p	Volume of each peptide
V_{cell}	Volume of each cell
w	Binding energy of a peptide on membranes
w_B (w_H)	Binding energy of a peptide on bacterial (host) cell membranes
u	Trapping energy of a peptide in a cell
u_B (u_H)	Trapping energy of a peptide in a bacterial (host) cell

Section 4 summaries the results for peptide activity and selectivity as a function of cell density; the effect of peptide trapping is highlighted, and membrane selectivity and cell selectivity are compared. All the symbols and acronyms are defined in Table 1.

2. Cell and membrane selectivity of antimicrobial peptides

In this section, we present a pedagogical approach to peptide activity and selectivity, which shows how peptide selectivity depends on cell density and peptide trapping in cells. We start with a homogeneous system of either bacterial or host cells, referred to as a noncompetitive case, and turn to a mixture of both types of cells, referred to as a competitive case.

Before proceeding further, we introduce several parameters relevant for peptide activity and selectivity. A key "extrinsic" parameter is the number density of peptides, denoted as C_p; so is the density of cells, C_{cell}.[12,14,17,18] The surface area of each cell, A_{cell}, matters.[17] In terms of the number of membrane-bound peptides, doubling A_{cell} for given C_{cell} is equivalent to doubling C_{cell} for given A_{cell}. The peptide selectivity arises primarily from the difference in binding energy, denoted as w, between bacterial membranes and host-cell membranes.[1,16–18] Membrane rupture occurs in an all-or-none C_{cell}-dependent manner.[19,20,23] Recall that P/L is the molar ratio of membrane-bound peptides to lipids. At a certain value of C_p, *i.e.*, C_p^*, P/L reaches a threshold value required for membrane rupture, P/L^*;[10,19–21] C_p^* is either MIC or MHC. Finally, N_p denotes the number of trapped peptides per cell. This needs to be taken with caution. Below C_p^*, we assume that $N_p = 0$. In this case, penetration of peptides into a cell is expected to be a rare event, since it involves overcoming a large free energy barrier for crossing an otherwise intact cell membrane. At C_p^*, half of the cell membranes are ruptured. Thus, N_p can be interpreted as the number of peptides trapped in each dead cell. Alternatively, it can be considered as the "average" number of peptides trapped per cell at $C_p^* : N_p^*$. Here, we employ this definition of N_p, which is half of the number of trapped peptides in a dead cell. Beyond, N_p can be larger than N_p^*. But we ignore the possible weak dependence of N_p on C_p (see Section 2.2 for further discussion). As a result, for $C_p \geq C_p^*$, we use N_p and N_p^* interchangeably, unless otherwise indicated. Finally, the subscript 'B' or 'H' will be used to refer to bacteria and host cells, respectively, as in N_{pB}, N_{pH}, $(P/L)_B^*$, and $(P/L)_H^*$ (see Table 1). Similarly, C_B is the bacterial cell density and A_B is the bacterial cell surface area; a_B and a_H are the lipid headgroup

area of bacterial and host-cell membranes, respectively; the binding energy w_B and w_H can be interpreted similarly.

2.1 Homogeneous case

Fig. 2 illustrates how C_p^* depends on cell density C_{cell} in a homogeneous or noncompetitive case, consisting of either bacteria or host cells. Here, two concentric circles represent cells (membrane bilayers enclosing cells), whereas small circles stand for peptides; if filled ones are free or trapped, unfilled ones are membrane-bound. The fraction of bound peptides is controlled by the balance between entropy and energy[24] (also see ref. 25). At a low peptide concentration, peptides are mostly free, because of a large entropic penalty for binding even in a single-cell limit (Fig. 2(i)). As the peptide concentration C_p increases, the balance is swayed toward energy, which favors binding. As a result, the surface coverage of peptides P/L (molar ratio of bound peptides to lipids) also increases. Eventually, C_p reaches C_p^* (either MIC or MHC), at which $P/L = P/L^*$. Even in the single cell limit shown in (i), $C_p^* > 0$.

As the cell density increases, different cells compete for peptides. Even though the binding is driven by energy, this competition is entropic in origin and does not involve cell–cell interactions. This is responsible for the cell-density dependence of C_p^*. It can be worked out progressively as shown in Fig. 2. Now imagine introducing a second cell in Fig. 2(i), converting the system into the one in Fig. 2(ii). Because of the presence of the first cell, there will be less peptides for the second one: at $C_p = C_p^*$, the number of peptides the first cell consumed is equal to $[(P/L)^* \times A_{cell}/a_l + N_p]$, where a_l is the area of each lipid; recall A_{cell} is the surface area of each cell. The presence of a second cell in (ii) is equivalent to removing $[(P/L)^* \times A_{cell}/a_l + N_p]$ peptides in (i), which is at C_p^*. In order to remain at P/L^*, an extra number of peptides should be supplied. The required number of peptides is equal to $[(P/L)^* \times A_{cell}/a_l + N_p]$. This will raise C_p^* by $[(P/L)^* \times A_{cell}/a_l + N_p]/V$, where V is the volume of the system:

$$C_p^*(2\ \text{cells}) = C_p^*(1\ \text{cell}) + \left[\left(\frac{P}{L}\right)^* \frac{A_{cell}}{a_l} + N_p\right] \frac{1}{V}. \quad (1)$$

Here, $C_p^*(1\ \text{cell})$ is either MIC or MHC in the single-cell case.

The progression from (i) to (iii) shows how this analysis can be extended to the N_{cell}-cell case:

$$\begin{aligned} C_p^* &= C_p^*(1\ \text{cell}) + \left[\left(\frac{P}{L}\right)^* \frac{A_{cell}}{a_l} + N_p\right] \frac{(N_{cell}-1)}{V} \\ &\approx C_p^*(1\ \text{cell}) + \left[\left(\frac{P}{L}\right)^* \frac{A_{cell}}{a_l} + N_p\right] C_{cell}, \end{aligned} \quad (2)$$

where the second equality holds if $N_{cell} \gg 1$, as is often the case. This equation can be applied to a homogeneous system of either bacteria or host cells.

Eqn (2) becomes

$$\text{MIC}(C_B) = \text{MIC}_0 + \left[\left(\frac{P}{L}\right)_B^* \frac{A_B}{a_B} + N_{pB}\right] C_B \quad (3a)$$

$$\text{MHC}(C_H) = \text{MHC}_0 + \left[\left(\frac{P}{L}\right)_H^* \frac{A_H}{a_H} + N_{pH}\right] C_H. \quad (3b)$$

Here MIC_0 and MHC_0 are, respectively, the MIC and MHC in the low-cell density limit: $C_p(C_{cell} \to 0) \approx C_p^*(1\ \text{cell})$.

Eqn (3) can be viewed as a function of C_{cell}: C_B or C_H. Both the MIC and the MHC increase linearly with the cell density C_B and C_H, respectively. The slope of the relation in eqn (3), $[(P/L)^* A_{cell}/a_l + N_p]$, is the total number of peptides consumed per cell

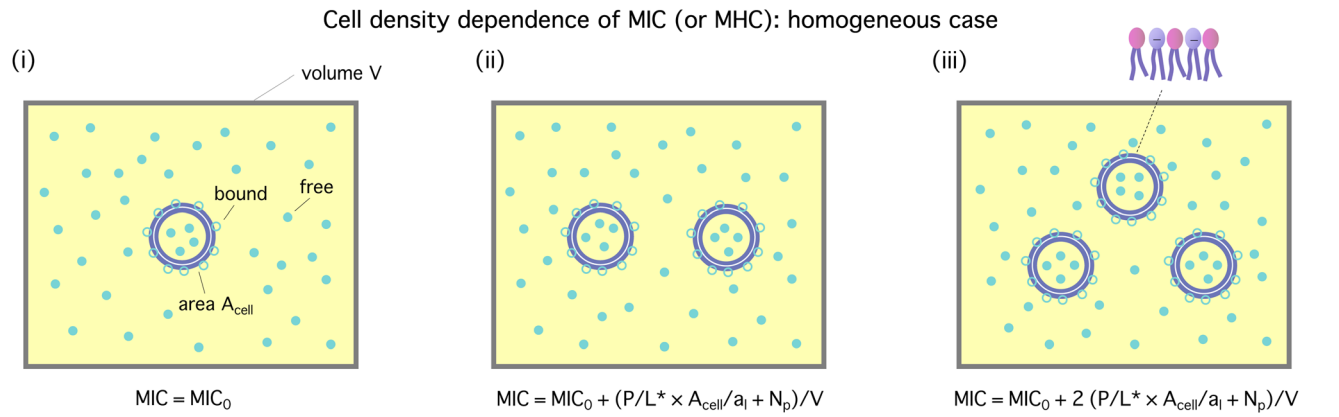

Fig. 2 Cell-density dependence of C_p^*, *i.e.*, either MIC or MHC: a homogeneous or noncompetitive case. Cells are represented by two concentric circles and peptides by filled (free or trapped) or unfilled circles (membrane-bound). As the peptide concentration C_p increases, their surface coverage P/L (molar ratio of peptides to lipids) also increases and eventually reaches a threshold P/L^* at C_p^*. Even in the single-cell limit shown in (i), $C_p^* > 0$, because of the entropy of peptides, which favors unbinding. Imagine introducing a second cell in (i), converting the system into the one in (ii). The number of peptides the first cell consumed is equal to $(P/L^* \times A_{cell}/a_1 + N_p)$, where a_l is the area of each lipid. In order to remain at P/L^*, the same number of peptides should be supplied. This will raise C_p^* by $P/L^* \times A_{cell}/a_l + N_p/V$, where V is the volume of the system: $C_p^*(2\ \text{cells}) = C_p^*(1\ \text{cell}) + (P/L^* \times A_{cell}/a_l + N_p)/V$. The progression from (i) to (iii) shows that $C_p^* = C_p^*(1\ \text{cell}) + (N_{cell} - 1) \times (P/L^* \times A_{cell}/a_l + N_p)/V \approx C_p^*(1\ \text{cell}) + (P/L^* \times A_{cell}/a_l + N_p) \times C_{cell}$. When applied to bacteria, this equation become $\text{MIC}(C_{cell}) = \text{MIC}_0 + (P/L^* \times A_{cell}/a_l + N_p)C_{cell}$, where MIC_0 is MIC in the low-cell density limit: $C_{cell} \to 0$. Figure adapted with permission from ref. 17. Copyright 2015 American Chemical Society; Reproduced with modifications from ref. 18 with permission from the Royal Society of Chemistry.

at $P/L = (P/L)^*$. This is larger for larger N_p; peptide trapping in cells makes C_p^* increase more rapidly with C_{cell}. The 'y'-axis intercept, either MIC_0 or MHC_0, is set by the interaction of peptides with membranes among others (see Section 3). The value of P/L^* reflects membrane curvature (peptide parameters as well).[19–21] It is larger for PE (phosphatidylethanolamine)-containing bacterial membranes, which tend to develop a negative curvature. However, this does not change P/L^* by an order of magnitude. For the peptide melittin, for instance, $(P/L)_B^* \approx 0.02$ and $(P/L)_H^* \approx 0.01$.[19–21]

Imagine combining MHC and MIC values obtained separately for homogeneous solutions. The ratio MHC/MIC increases with C_H: the larger C_H is, the larger the selectivity is. As evidenced below, this does not correctly represent the selectivity in a biological-relevant medium (*e.g.*, a mixture of host cells and bacteria) but tends to overestimate it.

2.2 Competitive case

The homogeneous-case analysis in Fig. 2 can be extended to a mixture of bacterial and host cells, referred to as a competitive case, as shown in Fig. 3. If the concentric circles in blue represent bacterial cells, the pink ones stand for the host cells. Fig. 3(i) shows a single bacterial cell at the MIC. The introduction of a host cell in Fig. 3(ii) will reduce the amount of peptides for the bacterial cell. The extra number of peptides to maintain C_p at the MIC is equal to $[(P/L)_H \times A_H/a_H + N_{pH}]$; similarly, in Fig. 3(iii), the number of peptides that should be added is $[(P/L)_B^* \times A_B/a_B + N_{pB}] + 2[(P/L)_H \times A_H/a_H]$

The progression from (i) to (iii) suggests that

$$\mathrm{MIC}(C_B, C_H) = \mathrm{MIC}_0 + \left[\left(\frac{P}{L}\right)_B^* \frac{A_B}{a_B} + N_{pB}\right] C_B + \left(\frac{P}{L}\right)_H \frac{A_H}{a_H} C_H \quad (4a)$$

$$\mathrm{MHC}(C_B, C_H) \stackrel{?}{=} \mathrm{MHC}_0 + \left[\left(\frac{P}{L}\right)_H^* \frac{A_H}{a_H} + N_{pH}\right] C_H + \left[\left(\frac{P}{L}\right)_B \frac{A_B}{a_B} + N_{pB}\right] C_B. \quad (4b)$$

If N_p is set to zero as for model membranes, the second equation in eqn (4) can be obtained from the first one by

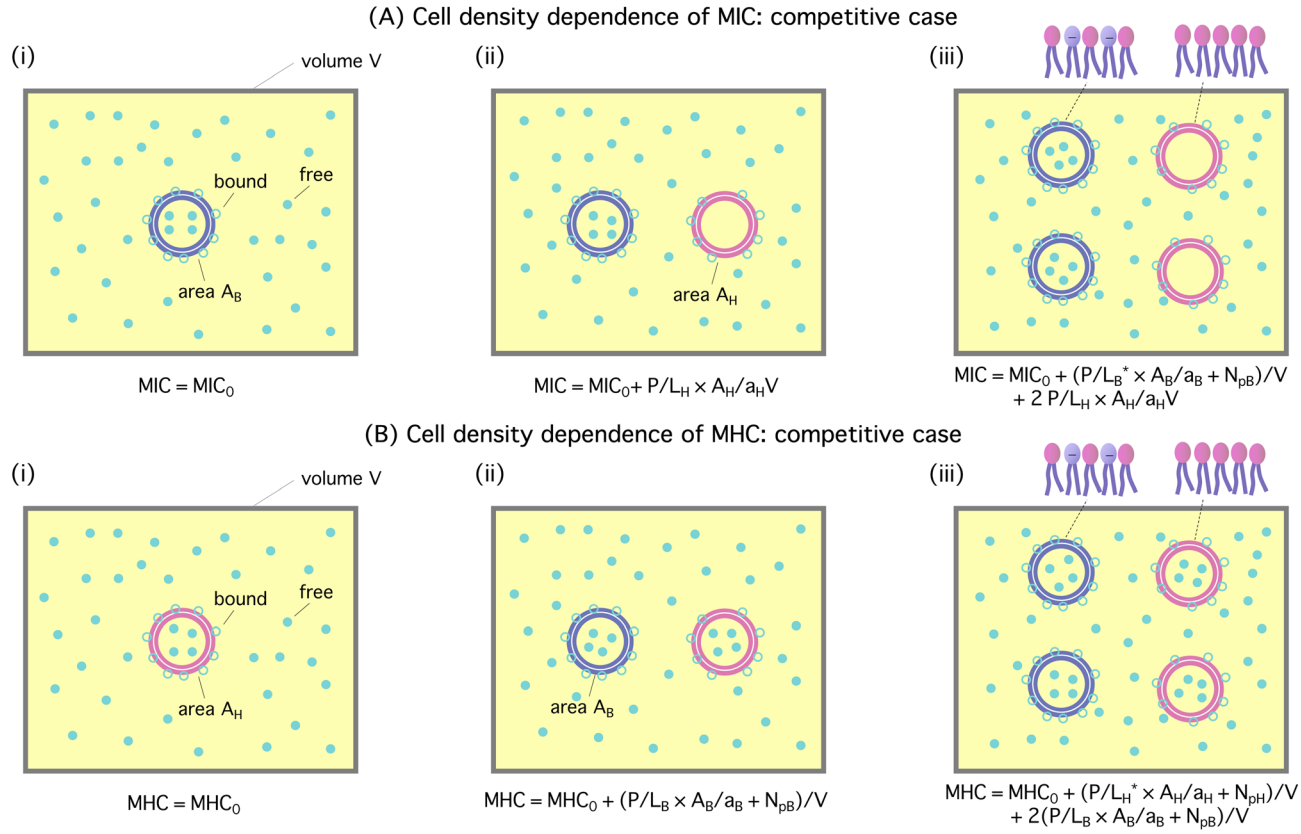

Fig. 3 Cell-density dependence of MIC (A) and MHC (B): competitive case. Cells are represented by two concentric circles and peptides by filled (free or trapped) or unfilled circles (membrane-bound); if the blue circles represent bacterial cells, the pink ones stand for host cells. Let $A_{cell} = A_B$ or A_H be the bacterial or host cell surface area, respectively; a_B and a_H the lipid headgroup area of the bacterial or host-cell membranes, respectively; N_{pB} and N_{pH} the number of trapped peptides in each bacterial and host cell, respectively; $(P/L)_B$ and $(P/L)_H$ are the molar ratio of bound peptides to lipids on the bacterial and host-cell membranes, respectively. (A) The progression from (i) to (iii) suggests that $\mathrm{MIC}(C_{cell}) = (\mathrm{MIC})_0 + [A_B/a_B \times (P/L)_B^* + N_{pB}]C_B + A_H/a_H \times (P/L)_H C_H$. (B) Using a similar line of reasoning, we arrive at $\mathrm{MHC}(C_{cell}) = (\mathrm{MHC})_0 + [A_H/a_H \times (P/L)_H^* + N_{pH}]C_H + [A_B/a_B \times (P/L)_B + N_{pB}]C_B$. Figure adapted with permission from ref. 17. Copyright 2015 American Chemical Society; Reproduced with modifications from ref. 18 with permission from the Royal Society of Chemistry.

swapping the role of bacteria with that of host cells. Here $(P/L)_H$ in eqn (4a) is the surface coverage of peptides on the host cells evaluated at $C_p = \text{MIC}$, whereas $(P/L)_B$ in eqn (4b) is the surface coverage of peptides on bacteria evaluated at $C_p = \text{MHC}$.

Note that these two lines of equations in eqn (4) are not fully symmetric with respect to the exchange in role between host cells and bacteria for the obvious reason: as C_p increases, the MIC will be reached first. This explains why the last term in eqn (4a) does not contain N_{pH}. In other words, $(P/L)_H < (P/L)_H^*$ in eqn (4a). In contrast, $(P/L)_B > (P/L)_B^*$ in eqn (4b). As a result, over a sizeable C_p range, the peptide under consideration is active against bacteria only and is thus selective.

Also, eqn (4b) needs to be understood with caution. Beyond $(P/L)_B^*$, some of bound peptides start to rupture the membranes by forming pores, for instance. The last term in these equations may be interpreted as the total amount of bound peptides whether on the membrane surface or in pores. As a result, the binding energy w_B needs to be interpreted accordingly. As it turns out, the term inside [...] in eqn (4) is dominated by N_p (see below). Furthermore, w_H, which governs peptide binding and influences $(P/L)_H$, is not constant but can vary with $(P/L)_H$. The main source of this dependence is the electrostatic interaction between bound peptides. But this dependence is generally weak, since the distance between bound peptides for $(P/L) \leq (P/L)^* \approx 0.01$ is typically larger than the Debye screening length, r_D, beyond which the electrostatic interaction is exponentially screened.[25] At $(P/L)^* = 0.01$, the typical distance between the adjacent peptides is $\approx \sqrt{100 \times 70}\text{Å} \approx 80\text{Å}$. This is appreciably larger than the screening length under physiological conditions (*e.g.*, in the presence of 100 mM of monovalent salts): $r_D \approx 10$ Å. Finally, in eqn (4b), N_{pB} is the number of trapped peptides in each cell above the MIC. The value of this parameter will eventually be determined by chemical equilibrium between trapped peptides and those on the membrane or in the bulk. The energetics of this is unknown and can be influenced by a number of factors such as peptide's interaction with cellular components and crowding in the cell. As mentioned in Section 2, in our consideration, we ignore this complexity and approximate N_{pB} in eqn (4b) by N_{pB}^*, *i.e.*, N_{pB} at MIC.

It is worth noting that eqn (3) and (4) are a special case of the following relations:

$$C_p = \frac{1}{v_p} \times \frac{\frac{A_p}{a_B}\left(\frac{P}{L}\right)_B}{1 - \frac{A_p}{a_B}\left(\frac{P}{L}\right)_B} e^{w_B/k_BT} + \left[\left(\frac{P}{L}\right)_B \frac{A_B}{a_B} + N_{pB}\right] C_B + \left[\left(\frac{P}{L}\right)_H \frac{A_H}{a_H} + N_{pH}\right] C_H \quad (5a)$$

$$C_p = \frac{1}{v_p} \times \frac{\frac{A_p}{a_H}\left(\frac{P}{L}\right)_H}{1 - \frac{A_p}{a_H}\left(\frac{P}{L}\right)_H} e^{w_H/k_BT} + \left[\left(\frac{P}{L}\right)_H \frac{A_H}{a_H} + N_{pH}\right] C_H + \left[\left(\frac{P}{L}\right)_B \frac{A_B}{a_B} + N_{pB}\right] C_B. \quad (5b)$$

Here, v_p is the volume occupied by each peptide in the bulk and A_p is the peptide area on the membrane surface. The first term in each line is inspired by eqn (11); recall w_B and w_H are, respectively, the binding energy of a given peptide on bacterial and host-cell membranes (for details, see the ESIof ref. 17 or Section 3). If evaluated at $(P/L)_B = (P/L)_B^*$, the first term on the right hand side of eqn (5a) is MIC_0; the first term in eqn (5b) can be interpreted similarly. Strictly speaking, both w_B and w_H have a weak dependence on P/L. At the relevant range of P/L around P/L^*, however, this dependence can be neglected as discussed above.

The meaning of N_p in eqn (5) is somewhat different from that in eqn (4). As noted above, eqn (5) is more general in the sense that C_p on the left hand side does not have to be equal to C_p^*, which is either MIC or MHC. As a result, N_p in eqn (5) varies with P/L and is generally different from N_p^*; $N_p = N_p^*$ for $(P/L) = (P/L)^*$ and $N_p = 0$ for $(P/L) < (P/L)^*$ Accordingly, $N_{pH} = 0$ in eqn (5a), when $C_p = \text{MIC}$ (<MHC). Eqn (5a) then reduces to the MIC expression in eqn (4a). Similarly, eqn (5b) becomes the MHC expression in eqn (4b) in an appropriate limit.

For given values of C_p and cell density (C_B and C_H), the two equations in eqn (5) can be solved simultaneously for P/L: $(P/L)_B$ and $(P/L)_H$. Initially, we set $N_p = 0$ and increase C_p gradually from zero. At some value of C_p, $(P/L)_B$ reaches $(P/L)_B^*$. The resulting value of C_p with N_{pB} set to N_{pB}^* is the MIC. We then increase C_p further until $(P/L)_H = (P/L)_H^*$. The resulting C_p with $N_{pH} = N_{pH}^*$ is the MHC. In this step, $(P/L)_B$ in eqn (5) is larger than $(P/L)_B^*$. In reality, pore formation in bacterial membranes can complicate the energetics of peptide binding to the membrane. But this complication will not change the MHC in any significant way, since $(P/L)_B (A_B/a_B) \ll N_{pB}$ at or above the MHC, as discussed in ref. 16 (also see below); the main source of inoculum effects is the trapping of peptides in cells rather than peptide adsorption to membranes. For model membranes, however, this reasoning is not applicable. In our coarse-grained model, all the details governing peptide binding are subsumed into the parameter w (w_B and w_H). As noted above, w has a weak dependence on P/L and can also be influenced by pore formation. In a Langmuir-type model such as the one employed here, w is often approximated by its representative value. With a similar spirit, we will use a standard value of N_p, as discussed in Section 3.

Let's analyze the relative significance of peptide trapping in determining the cell-density dependence of MIC or MHC. For this, we essentially repeat the earlier analysis in ref. 16. Compare the two terms with each other inside [...] in eqn (4): the number of membrane-bound peptides and the number of absorbed peptides per cell. For the representative bacterium *E. coli*, $A_B \approx 12\ \mu\text{m}^2$, which is twice the area of the inner or outer layer of the cytoplasmic membrane.[16,17] Since $a_B \approx a_H \approx 70\ \text{Å}^2$, $A_B/a_B \approx 1.7 \times 10^7$. For the peptide melittin, $(P/L)_B^* \approx 0.02$ and $(P/L)_H^* \approx 0.01$.[19–21] We thus find $(P/L)_B^*(A_B/a_B) \approx 3.4 \times 10^5$. This number is much smaller than $N_{pB} \approx 10^7$ to 10^8.[14] For the outer *E. coli* membrane, $(P/L)_B^*$ is several fold larger,[10,26] but this does not change the picture. For human red blood cells as representative host cells, $A_H \approx 17A_B$ and $A_H/a_H \approx 2.9 \times 10^8$. As a result, we obtain $(P/L)_H^*(A_H/a_H) \approx 2.9 \times 10^6$. This is smaller

than $N_{pH} \approx 10^7$.[12,13] The main source of inoculum effects is the trapping of peptides inside dead cells at or above $(P/L)^*$.

The analysis above implies that only the last term in eqn (4a) has a noticeable, explicit dependence on the binding energy w_H for given MHC_0. As a result, the MIC in eqn (4a) can be sensitive to w_H, whereas the MHC in eqn (4b) is not. For similar reasons, both the MIC and the MHC in eqn (4) and (3) are not sensitive to w_B for a fixed value of MIC_0. For the homogeneous case in eqn (3), none of the MIC and the MHC is "explicitly" sensitive to w_B or w_H.

Similarly to what was observed in the homogenous case in Section 2.1, peptide trapping in cells (the main inoculum effect) makes C_p^* increase more rapidly with C_{cell}. It makes steeper the slope of a C_p^* curve *versus* C_{cell}.

2.3 Limiting cases

It proves instructive to take some mathematical limits and simplify eqn (4). First, consider the case $C_B = C_H$. In the low cell-density limit, *i.e.*, $C_B = C_H \to 0$, the MIC and MHC in eqn (4) reduce to MIC_0 and MHC_0, respectively, as there is no competition between different cells (or membranes) to bind peptides. As a result, the distinction between the competitive and noncompetitive cases disappears in this limit.

In the high-cell-density case, for simplicity, let's assume that $A_B = A_H$ and $N_p = 0$, as is often the case for lipid bilayers, and $a_B = a_H \approx 70$ Å^2, which is a good approximation (if $A_H \neq A_B$, this analysis is applicable to the case: $A_BC_B = A_HC_H$). The competitive selectivity, MHC/MIC, becomes cell-density independent: $MHC/MIC \approx [(P/L)_B + (P/L)_H^*]/[(P/L)_B^* + (P/L)_H] \gtrsim 1$. To understand the origin of the inequality, note that $(P/L)_B$ in the numerator is larger than $(P/L)_B^*$ in the denominator, whereas $(P/L)_H$ in the denominator is smaller than $(P/L)_H^*$ in the numerator. Thus MHC/MIC in this limit will get saturated at some constant larger than 1.

In the noncompetitive case with $C_B = C_H$, however, the ratio MHC/MIC approaches the following constant: $(P/L)_H^*/(P/L)_B^*$. The threshold P/L is better known for lipid bilayers mimicking cell membranes than for cell membranes. As noted in Section 2.1, because of the presence of PE (phosphatidylethanolamine) in bacterial cell-membrane mimics, $(P/L)_B^*$ is somewhat larger than $(P/L)_H^*$. In the large cell-density limit in the noncompetitive case, we thus have MHC/MIC $\lesssim 1$. There is a noticeable difference between the competitive and noncompetitive cases in the large cell-density limit; the selectivity is higher in the former case.

If $C_H \gg C_B$, eqn (4) can be simplified as $MIC \approx (MIC)_0 + A_H/a_H \times (P/L)_H C_H$ and $MHC \approx (MHC)_0 + A_H/a_H \times (P/L)_H^* C_H$. Note that the MIC in this case is much larger than the MIC for the corresponding bacteria-only case and the MHC here is approximately equal to the MHC for the corresponding host-cell-only case, as illustrated in Fig. 4. Accordingly, the ratio MHC/MIC is roughly independent of C_B and approaches a constant of order 1, as $C_H \to \infty$ (while C_B is held fixed).

Imagine combining two sets of data: one set for bacteria only and one set for host cells only, *i.e.*, two homogeneous cases in eqn (3). If $C_H \gg C_B$, MHC/MIC $\to \infty$ as $C_H \to \infty$. This limiting behavior in the homogeneous case is opposite to the one obtained for the corresponding competitive case (see Fig. 4). It explains how the selectivity can be excessively overestimated.

When $N_p \neq 0$ and $A_B \neq A_H$, our analysis should reflect these inequalities. But the difference caused by them is often quantitative rather than qualitative, as evidenced in Section 4.

A full analysis of eqn (4) is involved. As discussed earlier,[16] in some relevant limits, we can simplify eqn (4) (see ref. 16). This is particular the case for $C_H \gg C_B$ as in whole blood. In this case, eqn (4) can be approximated as

$$MIC(C_B, C_H) \approx MIC_0 + \left(\frac{P}{L}\right)_H \frac{A_H}{a_H} C_H \tag{6a}$$

$$MHC(C_B, C_H) \approx MHC_0 + \left[\left(\frac{P}{L}\right)_H^* \frac{A_H}{a_H} + N_{pH}^*\right] C_H. \tag{6b}$$

Here, $(P/L)_H$ is to be evaluated at $C_p = MIC$.

Notice the obvious difference the competitive MIC in eqn (6a) and the noncompetitive one in eqn (3a). As discussed earlier in Section 2.3 and in Fig. 4, for $C_H \gg C_B$, the competitive MIC is much larger than the noncompetitive one. In contrast, the MHC is approximately the same for both cases. This results

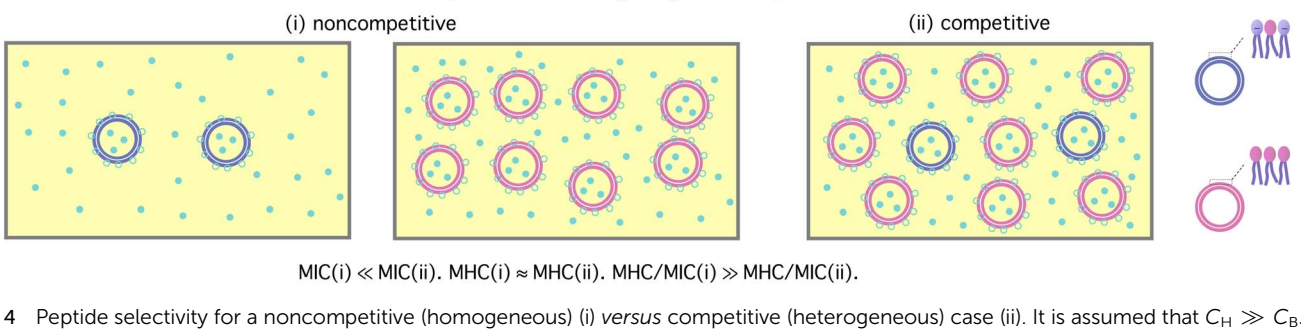

Fig. 4 Peptide selectivity for a noncompetitive (homogeneous) (i) *versus* competitive (heterogeneous) case (ii). It is assumed that $C_H \gg C_B$. In this case, whether the selectivity is measured noncompetitively (i) or competitively (ii) has a profound impact on the selectivity. It can be excessively overestimated in the noncompetitive case (i) with reference to the corresponding competitive case (ii), since the MIC is much larger for the latter case. The opposite is true if $C_H \ll C_B$. Figure adapted with permission from ref. 17. Copyright 2015 American Chemical Society; Reproduced with modifications from ref. 18 with permission from the Royal Society of Chemistry.

in much larger selectivity in the noncompetitive case compared to the corresponding competitive case. This finding is consistent with the analysis above with N_p set to zero.

For the case $C_H \gg C_B$, the ratio MHC/MIC becomes

$$\frac{\mathrm{MHC}}{\mathrm{MIC}} \approx \frac{\mathrm{MHC}_0 + \left[\left(\frac{P}{L}\right)_H^* \frac{A_H}{a_H} + N_{pH}^*\right] C_H}{\mathrm{MIC}_0 + \left(\frac{P}{L}\right)_H \frac{A_H}{a_H} C_H}. \quad (7)$$

Eqn (7) suggests that peptide trapping in the host cells enhances peptide selectivity; it works in favor of the host cells. This is a natural consequence of the MHC that increases with N_{pH}^*. Since the second term inside […] in the numerator of eqn (7) is larger than the first term roughly by an order of magnitude (see Section 2.2), the effect of peptide trapping on the selectivity is up to about 10-fold.

So far, we have used a simple biophysical picture, based on Fig. 2–4 to explore how peptide selectivity depends on cell density (C_B or C_H) and on the way it is measured (*i.e.*, competitive *versus* noncompetitive). The *y*-intercepts, MIC_0 and MHC_0, may be considered as fitting parameters. They can also be related to more microscopic parameters. In the next section, we recapture the main results in this section; we then relate MIC_0 and MHC_0 to the biophysical parameters of peptides and membranes.

3. Langmuir binding model

In this section, using a Langmuir-type model for molecular binding,[25] we derive the main results presented in Section 2 and relate MIC_0 and MHC_0 to the biophysical parameters of peptides and membranes. Note that such a model was already considered recently.[17,18] Here, we recapture the essence of this consideration and generalize it to include peptide trapping in a cell. It suffices to focus on the homogeneous case, since the dependence of peptide activity on cell density in the competitive case is already obvious from eqn (4).

In this model, peptides are either "free" (in the bulk) or "bound"; bound peptides are further classified as adsorbed to the cell surface or trapped inside a cell (see Fig. 2); trapped ones can bind to intracellular components. Initially, peptide binding occurs on the outer membrane layer or the outmost one in the case of Gram-negative bacteria. Adsorbed peptides will be eventually symmetrically distributed between the two layers after or even prior to membrane rupture[27,28] (also see ref. 17). For simplicity, we ignore peptide trapping below C_p^* within typical experimental time scales. Indeed, it was shown that a large amount of trapped peptides were observed in dead bacterial cells, but not in dividing cells.[14] At and beyond C_p^*, the amount of bound peptides is determined by chemical equilibrium between free and bound states.

Let w and u be the adsorption and trapping energy, respectively. The value of w is typically more negative for bacterial membranes containing a large fraction of anionic lipids. It is worth noting that w is an effective parameter in which the effect of lipid demixing and peptide–peptide interactions on the membrane surface are subsumed (see ref. 18 for details). Similarly, u takes into account the interactions of trapped peptides with intracellular components as well as their mutual interactions inside the cell; it is also influenced by molecular crowding in the cell.[29,30]

Let C_p be the total concentration of peptides whether free or bound, σ_p [$=(P/L)/a_l$] the planar density of adsorbed peptides and A_p the area occupied by a bound peptide; n_p the number density of trapped peptides, and v_p the volume of each peptide; $n_p = 0$ when $P/L < P/L^*$ and $n_p = N_p/V_{cell}$ when $P/L = P/L^*$, where V_{cell} is the volume of each cell. In our Langmuir model, the chemical potential of bound peptides μ_{bound} at and above P/L^* can readily be obtained as

$$\frac{\mu_{bound}}{k_B T} = \frac{w}{k_B T} + \ln\left(\frac{\sigma_p A_p}{1 - \sigma_p A_p}\right) = \frac{u}{k_B T} + \ln\left(\frac{n_p v_p}{1 - n_p v_p}\right). \quad (8)$$

Here and below, k_B is the Boltzmann constant and T the temperature. The logarithmic term is related to the number of ways in which bound peptides are distributed on the membrane surface or inside the cell. The second equality holds in chemical equilibrium between adsorbed and trapped peptides.

The chemical potential of free peptides is

$$\frac{\mu_{free}}{k_B T} = \ln\left\{\left[C_p - C_{cell}\left(\sigma_p A_{cell} + n_p V_{cell}\right)\right] v_p\right\}. \quad (9)$$

Note that the expression inside […] is the concentration of free peptides and the term inside (…) is the inoculum size.

By equating the two chemical potentials in eqn (8) and (9), we obtain

$$C_p = \frac{1}{v_p} \times \frac{\frac{A_p}{a_l}\left(\frac{P}{L}\right)}{1 - \frac{A_p}{a_l}\left(\frac{P}{L}\right)} e^{w/k_B T} + \left[\frac{A_{cell}}{a_l}\left(\frac{P}{L}\right) + n_p V_{cell}\right] C_{cell} \quad (10a)$$

$$C_p = \frac{1}{v_p} \times \frac{n_p v_p}{1 - n_p v_p} e^{u/k_B T} + \left[\frac{A_{cell}}{a_l}\left(\frac{P}{L}\right) + n_p V_{cell}\right] C_{cell}. \quad (10b)$$

In this final expression, we eliminated σ_p in favor of P/L *via* the relation $\sigma_p a_l = P/L$ (with a_l as the lipid head-group area). In the absence of peptide trapping in cells (or below C_p^*), eqn (10a) with $n_p = 0$ describes chemical equilibrium between free and adsorbed peptides; eqn (10b) becomes irrelevant.

At C_p^*, *i.e.*, either MIC or MHC,

$$C_p^* = \frac{1}{v_p} \times \frac{\frac{A_p}{a_l}\left(\frac{P}{L}\right)^*}{1 - \frac{A_p}{a_l}\left(\frac{P}{L}\right)^*} e^{w/k_B T} + \left[\frac{A_{cell}}{a_l}\left(\frac{P}{L}\right)^* + n_p^* V_{cell}\right] C_{cell} \quad (11a)$$

$$C_p^* = \frac{1}{v_p} \times \frac{n_p^* v_p}{1 - n_p^* v_p} e^{u/k_B T} + \left[\frac{A_{cell}}{a_l}\left(\frac{P}{L}\right)^* + n_p^* V_{cell}\right] C_{cell}. \quad (11b)$$

Here $n_p^* V_{cell} = N_p^*$ is the (average) number of peptides trapped in each cell at $C_p = C_p^*$.

Comparison between eqn (11) and (3) leads to the following relation

$$\mathrm{MIC}_0 = \frac{1}{v_p} \times \frac{\frac{A_p}{a_B}\left(\frac{P}{L}\right)^*_B}{1 - \frac{A_p}{a_B}\left(\frac{P}{L}\right)^*_B} e^{w_B/k_BT} = \frac{n^*_{pB}}{1 - n^*_{pB} v_p} e^{u_B/k_BT} \quad (12a)$$

$$\mathrm{MHC}_0 = \frac{1}{v_p} \times \frac{\frac{A_p}{a_H}\left(\frac{P}{L}\right)^*_H}{1 - \frac{A_p}{a_H}\left(\frac{P}{L}\right)^*_H} e^{w_H/k_BT} = \frac{n^*_{pH}}{1 - n^*_{pH} v_p} e^{u_H/k_BT}. \quad (12b)$$

Here the subscript 'B' and 'H' refer to bacteria and host cells, respectively. Both MIC_0 and MHC_0 are exponentially sensitive to w or u but they are not as sensitive to other quantities. This energy scale is the main origin of peptide selectivity. The results in eqn (12) can be used in eqn (4) (competitive) or in eqn (3) (noncompetitive).

It is worth mentioning that we will not attempt to solve eqn (10) for n_p, partly because the energetics involved in peptide trapping (*i.e.*, u) is not well known. Instead, we will use suitable values of $n_p V_{cell} = N_p \approx N^*_p$, the number of peptides trapped in each cell, inspired by recent experiments.[12–14] With this simplification, eqn (10) can readily be extended to the competitive case shown in Fig. 3. The cell-density dependence of C^*_p is already obvious in light of the discussion in Section 2.2; one can readily write down eqn (5).

4. Results

In this section, we present the results for peptide activity and selectivity obtained for model membranes (Section 4.1) and cells (Section 4.2). Recall that one of the main differences between the two comes from peptide trapping in the latter case. As detailed below, MIC_0 and MHC_0 are chosen differently for the two cases. If calculated values of these quantities are used for model membranes, they are chosen appropriately for cells.

4.1 Membrane selectivity

Following Section 2, we first present our results for peptide activity and selectivity without taking into account peptide trapping using peptide parameters relevant for a melittin-like peptide:[17,18] $(P/L)^*_B = 1/48 (P/L)_H = 1/99$,[19–21] $v_p = 33^3$ Å^3, and $A_p = 400$ Å^2.[17,18] For this peptide, w was mapped out for model membranes, mimicking bacterial and host-cell membranes: $w_B = -16.6 k_BT$ and $w_H = -6.72 k_BT$.[18] Also, $a_B = 71$ Å^2 (a_l for bacterial membranes), $a_H = 74$ Å^2 (a_l for host-cell membranes),[19–21] $A_B = 1.2 \times 10^9$ Å^2 $= 12$ μm^2 (suitable for *E. coli*), and $A_H = A_B$ or $A_H = 17A_B$ (as for human red blood cells).[17] Note here that this value of A_B is two times the surface area of *E. coli* (≈ 6 μm^2).[31] This is to reflect the symmetrical binding of peptides on the inner and outer layers of the cytoplasmic membrane, as discussed in Section 3. Finally, we set $N_p = 0$ as expected for model membranes. In reality, $N_p \neq 0$ at or beyond $(P/L)^*$. But practically, it can be set to zero, since the majority of peptides would remain 'free'; trapped peptides in model membranes are outnumbered by those in the bulk.

We have solved eqn (3) for the noncompetitive case and eqn (4) for the competitive case (both together with eqn (12)). This is equivalent to solving eqn (5) for P/L at and found C_p at which P/L is equal to P/L^*, as discussed below eqn (5). In Fig. 5, the resulting C^*_p, either MIC or MHC, as well as the ratio MHC/MIC are shown as a function of cell density: C_B or C_H. When C_H (C_B) is held fixed, the x axis represents C_B (C_H); for the case $C_H = C_B$, it indicates both C_H and C_B. If the competitive cases are represented by dashed lines with filled symbols, the noncompetitive ones are described by solid lines with open symbols.

The results in Fig. 5(A) and (B) suggest that both MICs and MHCs increase with increasing cell density (C_H or C_B), as expected from eqn (3) and (4). For $A_H = 17A_B$, the presence of a large amount of host-cell membranes ($C_H = 5 \times 10^9$ cells per mL) raises the MIC by an order of magnitude as long as $C_B \lesssim 5 \times 10^5$ cells per mL, compared to the case $C_H = 0$; for $A_H = A_B$, however, its impact on the MIC appears to be minor. There is no essential difference between the three cases in (A): (i) $C_H = C_B$, $A_H = A_B$, (ii) $C_H = 0$, $A_H = A_B$, and (iii) $C_H = C_B$, $A_H = 17A_B$ (labelled as (i), (ii), (iii), respectively, in the legend); in these cases, the MIC is insensitive to the presence of an equal amount of host-cell membranes or the value of A_H. As C_B increases, the MIC curves eventually collapse onto each other. In this case, it is dominated by the C_B-dependent term in eqn (4a). This applies to all the curves shown except the one in tangerine for which C_B is held fixed.

As shown Fig. 5(B), in the presence of a large amount of host-cell membranes ($C_H = 5 \times 10^9$ cells per mL), the MHC obtained with $A_H = 17A_B$ is about ten times larger than $\mathrm{MHC}_0 \approx 3$ μM (*i.e.*, the y-intercept of the curve labelled as (ii) or (ii')), as long as $C_B \lesssim 10^8$ cells per mL. Similarly, in the other cases shown, the MHC is larger for larger $A_H = 17A_B$ than for $A_H = A_B$, as long as $C_H \gtrsim 10^8$cells per mL. For this, compare a curve obtained with $A_H = A_B$ with the corresponding one obtained with $A_H = 17A_B$ (*e.g.*, the curves labelled as (i) and (i′) or those labelled as (ii) and (ii′)). The difference between (i) and (i′) seems somewhat minor, but the difference between (ii) and (ii′) (in the absence of bacterial membranes) is pronounced. Also, the MHC curve labelled as (i) lies somewhat above the one labelled as (ii), both obtained with $A_H = 17A_B$. In this case, the presence of an equal amount of bacterial membranes ($C_H = C_B$) increases slightly the MHC. When $A_H = A_B$ (see the curves labelled as (i′) and (ii′)), however, the presence of an equal amount of bacterial membranes has a more appreciable impact on the MHC. In other words, the presence of an equal amount of bacterial cells increases the MHC more effectively when $A_H = A_B$. This is consistent with eqn (3) or 4, which suggests that the MHC is more sensitive to C_B if A_H is smaller. The presence of 5×10^4 cells per mL of bacterial membranes ($A_H = 17A_B$) does not have any noticeable impact on the MHC (the data not shown for simplicity).

The MIC and MHC results in Fig. 5(A) and (B) suggest that the presence of an equal amount of bacterial membranes influences MHCs more effectively than the presence of an equal amount of host cell membranes influences MICs. For this, compare the two curves labeled as (i) $C_H = C_B$, $A_H = A_B$ and (ii) $C_H = 0$, $A_H = A_B$ in (A) as well as those labelled as (i') $C_H = C_B$, A_H

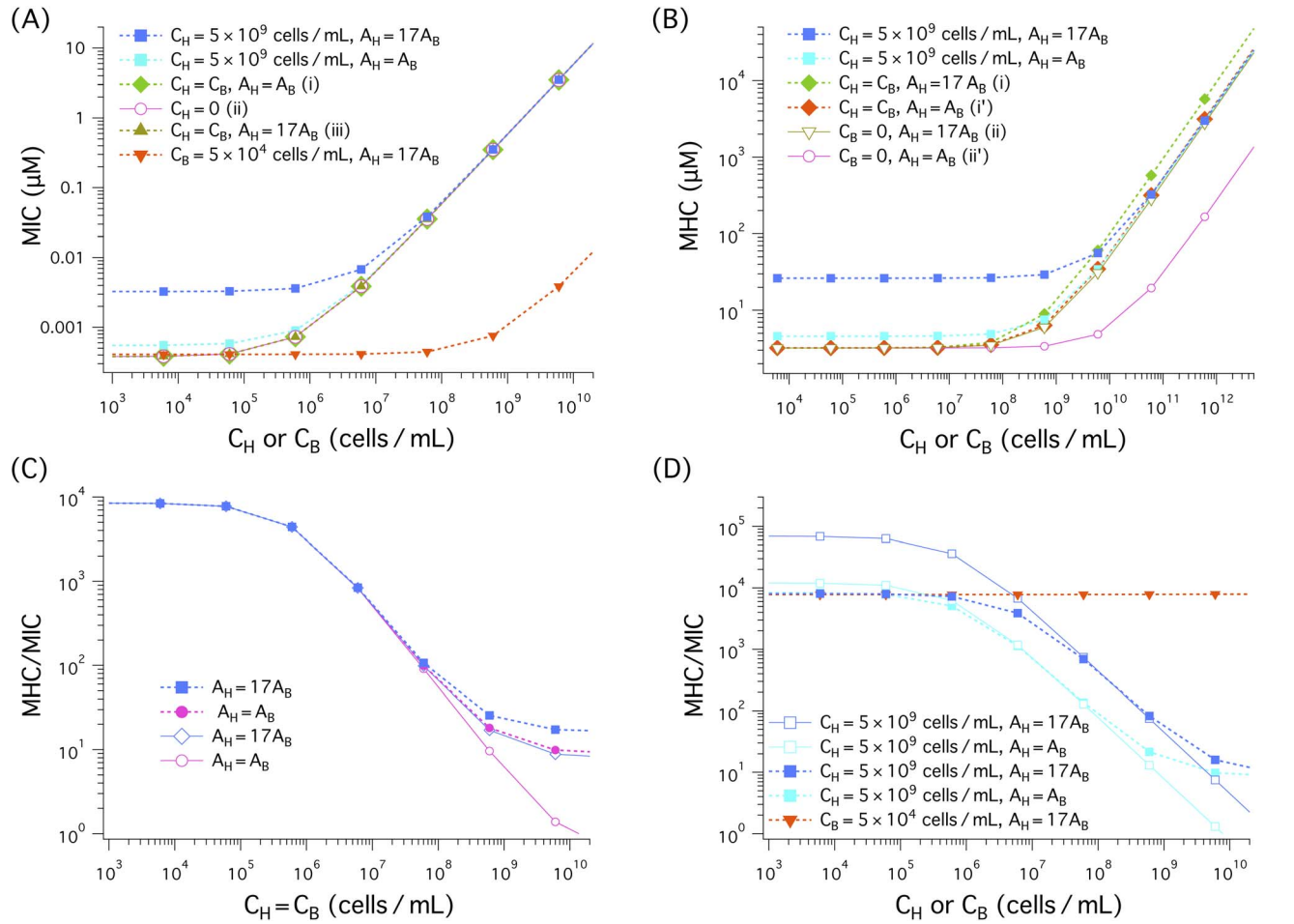

Fig. 5 Cell (membrane) density dependence of MIC, MHC, and MHC/MIC for the noncompetitive and competitive cases, represented by solid lines with unfilled symbols and dashed lines with filled symbols, respectively. When C_H (C_B) is held fixed, the 'x' axis represents C_B (C_H); for the case $C_H = C_B$, it stands for both C_H and C_B. We have chosen the parameter as follows: the bacterial cell surface area $A_B = 12$ μm^2 (suitable for *E. coli*); the host cell surface area $A_H = A_B$ and $A_H = 200$ μm^2 ≈ 17 × A_B (as for human red blood cells); $a_B = 71$ Å^2 and $a_H = 74$ Å^2; $P/L_B^* = 1/48$ and $P/L_H^* = 1/99$;[19–21] $v_p = 33^3$ Å^3 and $A_p = 400$ Å^2;[17,18] $w_B = -16.6\ k_BT$ and $w_H = -6.72\ k_BT$[18] as for the peptide melittin. (A)–(B) In all cases, both MICs and MHCs increase with increasing C_H or C_B, as expected from eqn (4a). Also, the presence of a large amount of hot-cell membranes ($C_H = 5 \times 10^9$ cells per mL) raises both the MIC and the MIC, almost by an order of magnitude for the case $A_H = 17A_B$ as long as $C_H \gg C_B$. There is no essential difference between the three cases labelled as (i), (ii), and (iii) in the legend in (A): the presence of an equal amount of host-cell membranes ($C_H = C_B$) or the value of A_H does not influence the MIC in any noticeable way. As shown in (B), the MHC is larger for larger A_H (*i.e.*, $A_H = 17A_B$). For this, compare a curve obtained with $A_H = A_B$ with the corresponding one obtained with $A_H = 17A_B$. Also, the MHC curve labelled as (i) lies somewhat above the one labelled as (ii), both obtained with $A_H = 17A_B$. In this case, the presence of an equal amount of bacterial membranes ($C_H = C_B$) increases slightly the MHC. When $A_H = A_B$ represented as (i') and (ii'), however, it has a more appreciable impact on the MHC. The competitive MHC in the presence of 5×10^4 cells per mL of bacterial membranes with $A_H = 17A_B$ is almost identical to the corresponding noncompetitive one (*i.e.*, $C_B = 0$) (data not shown for simplicity). The selectivity in (C), as measured by MHC/MIC, decreases as the membrane density increases; in both competitive and noncompetitive cases, we chose $C_H = C_B$. The difference between the competitive and noncompetitive cases becomes obvious when the cell density is $\gtrsim 10^8$ cells per mL, in which the selectivity is higher for the former case. Also the selectivity is higher for the larger A_H case as long as $C_B = C_H \gtrsim 10^8$ cells per mL. In (D), except for the red dashed curve with inverted filled triangles, $C_H = 5 \times 10^9$ cells per mL but C_B varies. Similarly to what the graph in (C) suggests, the selectivity in (D) decreases as C_B decreases. Compared to the competitive case represented by the blue dashed curve with filled squares, the corresponding noncompetitive case overestimates the selectivity by about one order of magnitude at a low C_B range of $C_B \lesssim 10^5$ cells per mL. The red dashed line with inverted triangles obtained with $C_B = 5 \times 10^4$ cells per mL is nearly flat in the C_H range shown.

$= A_B$ and (ii') $C_B = 0$, $A_H = A_B$ in (B). The two in (A) tend to collapse onto each other, whereas in (B) the curve obtained with $C_B = 0$, $A_H = A_B$ falls well below the other one. This difference can be attributed to the stronger binding of peptides to bacterial membranes.

In the competitive case with an excessive amount of host cells ($C_H = 5 \times 10^9$ cells per mL), however, the MIC in Fig. 5(A) and the MHC in Fig. 5(B) are much larger than in the other cases as long as $C_B \ll C_H$. This is consistent with what eqn (4) suggests: the presence of a large amount of host cells increases both the MIC and the MHC (see the relevant discussion in Section 2.3). These equation also suggest that the MIC or the MHC is generally larger for $A_H = 17A_B$ than for $A_H = A_B$, unless the A_H-independent terms dominate. For this, compare the two

curves in blue and cyan in (A) or (B), for instance. As discussed in Section 2, increasing A_H is equivalent to increasing C_H. This explains the observation of larger MIC and MHC values for larger A_H.[16,17]

The ratio MHC/MIC measures peptide selectivity. Our results for this ratio are shown in Fig. 5(C) and (D). In (C), $C_B = C_H$; in (D), except for the red dashed line, $C_H = 5 \times 10^9$ cells per mL but C_B is allowed to vary. In all cases, the selectivity decreases (or remains flat), as the cell density increases as discussed in Section 2. In (C), the difference between the competitive and noncompetitive cases for $A_H = A_B$ becomes obvious when the cell density is $\gtrsim 10^8$ cells per mL, in which the selectivity is higher for the former case. This is correlated with the observation that the MHC is higher for the competitive case in this range of cell density as shown in Fig. 5(B). Also, the selectivity is higher for the larger A_H case.

The competitive cases in (D), except for the red dashed line, contain a large amount of host cells ($C_H = 5 \times 10^9$ cells per mL) in addition to bacterial cells with variable C_B. In the noncompetitive measurement, MHCs obtained with the choice $C_H = 5 \times 10^9$ cells per mL were combined with MICs. Similarly to what the graphs in (C) suggests, the selectivity in (D) decreases as C_B increases. However, the selectivity in the noncompetitive case is overestimated compared to the corresponding competitive case, as long as $C_B \lesssim 10^7$ to 10^8 cells per mL $\ll C_H$. For the large A_H case, it is overestimated by up to an order of magnitude. When C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL, the (competitive) selectivity remains roughly flat in the C_H range shown.

This finding is well aligned with the view that the selectivity measured in a noncompetitive manner (with $C_H \gg C_B$) can be an experimental illusion.[11] This is not the case for the competitive selectivity. Even in the presence of a large amount of host cells, the selectivity measured in a competitive environment is not an experimental artifact. It just reflects the cell-density dependence of the selectivity, presented in Section 2.

4.2 Cell selectivity: inoculum effects

We have solved eqn (4) with realistic choices of N_p and mapped out various scenarios for peptide activity and selectivity. One of the challenges in this effort is that the parameters in these equations are not well known for real cells. In particular, w_B for Gram-negative bacteria is also influenced by the peptide interaction with their outer membrane (OM); recall that this is an effective parameter, in which microscopic details (*e.g.*, peptide charge, peptide interaction with the OM) are subsumed (see Section 3). This quantity has only recently been mapped out theoretically for the interaction of melittin-like peptides with model membranes.[18] For the reasons explained in Section 2, however, the dependence of peptide activity on w_B is reflected mainly through MIC_0. Furthermore, the MIC and the MHC in the homogeneous case in eqn (3) do not depend sensitively on w_B or w_H for given MIC_0 and MHC_0.

Here we do not attempt to calculate the effective binding energy w (either w_B or w_H) for real cells and use it in the computation of MIC_0 and MHC_0. Instead, we start with conveniently-chosen but biophysically-relevant values of MIC_0 and MHC_0: $MIC_0 = 1$ μm and $MHC_0 = 5$ μm (see ref. 14 and 15, for instance, for MIC_0). For simplicity, the number of trapped peptides N_p is chosen to be the same for bacteria and host cells: $N_p = 0, 10^7, 5 \times 10^7$. Otherwise, we choose the same parameters used in Fig. 5: the bacterial cell surface area $A_B = 12$ μm^2 (suitable for *E. coli*); the host cell surface area $A_H = 200$ μm$^2 \approx 17 \times A_B$ (as for human red blood cells); $a_H = 71$ Å^2 and $a_H = 74$ Å^2; $w_B = -16.6\ k_BT$ and $w_H = -6.72\ k_BT$;[18] $v_p = 33^3$ Å^3 and $A_p = 400$ Å^2.[17,18]

Fig. 6 displays the results for the MIC (A) and the MHC (B) for the noncompetitive and competitive cases, represented by solid lines with unfilled symbols or unfilled symbols and dashed lines with filled symbols, respectively. As in Fig. 5, when C_H (C_B) is held fixed, the 'x' axis represents C_B (C_H); for the case $C_H = C_B$, it stands for both C_H and C_B.

In all cases shown in Fig. 6(A) and (B), both MICs and MHCs increase linearly with increasing cell density (C_H or C_B), similarly to what is shown for model membranes in Fig. 5. This is a natural consequence of the cell-density dependence shown in eqn (4).

As indicated in the graph on the left in Fig. 6(A), the presence of an excess amount of host cells raises the MIC, more so for larger N_p as long as $C_B \lesssim 5 \times 10^8$ cells per mL; for this, compare the curve obtained with $C_H = 5 \times 10^9$ cells per mL with the one obtained with $C_H = 0$. Nevertheless, the MIC remains somewhat smaller than 10 μm if $C_B \lesssim 5 \times 10^7$ cells per mL. When $C_B \gtrsim 5 \times 10^8$ cells per mL, the presence of host cells does not have a significant impact on the MIC; in this case, peptide trapping in bacterial cells is a determining factor. For the same value of N_p, different curves representing different values of C_H collapse onto each other for sufficiently large C_B: $C_B \gtrsim 5 \times 10^8$ cells per mL. Also, the MIC obtained with $N_p = 5 \times 10^7$ increases more rapidly with cell density than the corresponding one obtained with $N_p = 10^7$ does, as suggested by eqn (4). Finally, for given N_p, there is no noticeable difference between the two cases: $C_B = C_H$ (competitive) and $C_H = 0$ (bacterial-cell only). The presence of an equal amount of host cells has an insignificant impact on the MIC. At the MIC, the host cells are above the MHC (no trapping in the cells) and their effect on the MIC is expected to be minor (see Section 2.2. for the relative significance of membrane association of peptides *versus* peptide trapping in cells).

As shown in the graph on the right in Fig. 6, when C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL, the MIC is insensitive to the value of N_p used, as if bacterial cells are in the low-cell density limit (*i.e.*, their presence creates a minimal inoculum effect). At the MIC, the host cells, which are present together with bacterial cells, are below the MHC. As a result, the binding of peptides to the host-cell membrane is responsible for the slow increase of the MIC with C_H. The presence of a large amount of bacterial cells ($C_B = 10^8$ cells per mL) increases the MIC about ten-fold as long as $C_H \lesssim 10^8$ cells per mL (the two homogenous MIC curves from the graph in the left are also included for comparison purposes).

Fig. 6(B) shows how the MHC varies as a function of cell density: C_B or C_H. In all cases, the MHC increases with increasing cell density. When $C_H = 5 \times 10^9$ cells per mL, the MHC is large and remains roughly flat as C_B increases up to C_B

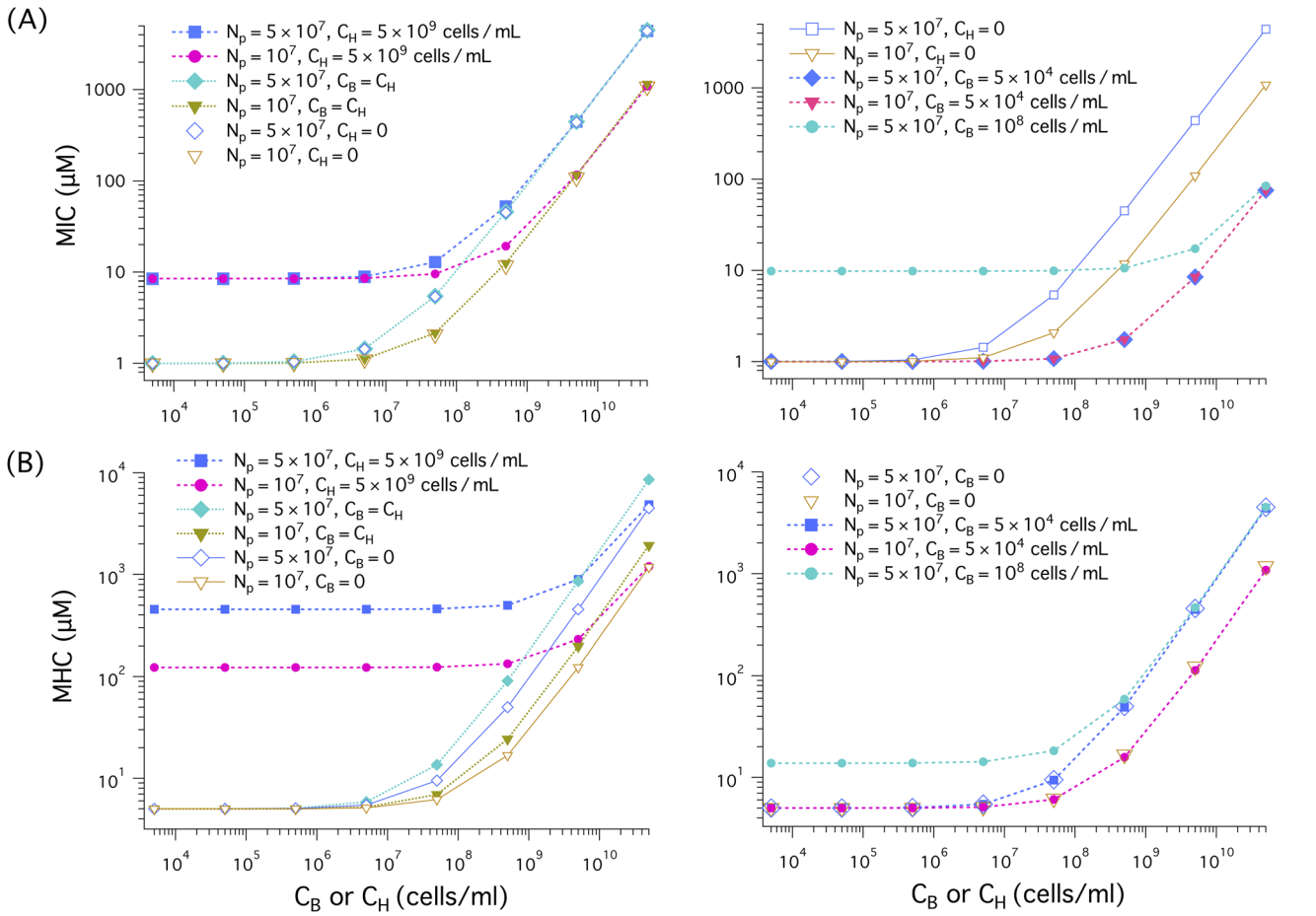

Fig. 6 Cell-density dependence of MIC (A) and MHC (B) for the noncompetitive and competitive cases, represented by solid lines with unfilled symbols (or unfilled symbols) and dashed lines with filled symbols, respectively. When C_H (C_B) is held fixed, the 'x' axis represents C_B (C_H); for the case $C_H = C_B$, it indicates both C_H and C_B (as indicated in the legends of (A), (B), and (C)). We have chosen the parameters as follows: $MIC_0 = 1$ μm and $MHC_0 = 5$ μm; $w_B = -16.6\ k_BT$ and $w_H = -6.72\ k_BT$, as for a melittin-like peptide; the bacterial cell surface area $A_B = 12\ \mu m^2$ (suitable for *E. coli*); the host cell surface area $A_H = 200\ \mu m^2 \approx 17 \times A_B$ (as for human red blood cells); the lipid headgroup area $a_B = 71\ Å^2$ and $a_H = 74\ Å^2$; $v_p = 33^3\ Å^3$ and $A_p = 400\ Å^2$. In all cases shown in (A) and (B), both the MIC and MHC increase with increasing cell density (C_H or C_B), as expected from eqn 4(a) and (b). (A) (Left) The presence of a large amount of host cells as in whole blood increases the MIC up to ten-fold, as long as $C_B \lesssim 5 \times 10^8$ cells per mL; for this, compare the curve obtained with $C_H = 5 \times 10^9$ cells per mL with the corresponding one obtained with $C_H = 0$. Also the MIC increases more rapidly, if N_p is larger. The MIC remains $\lesssim 10$ μm if $C_B \lesssim 5 \times 10^7$ cells per mL. If $C_B \gtrsim 5 \times 10^8$ cells per mL, the presence of host cells does not have a significant impact on the MIC; in this case, peptide trapping in bacterial cells is a determining factor. For the same value of N_p, different curves representing different values of C_H collapse onto each other for sufficiently large C_B: $C_B \gtrsim 5 \times 10^8$ cells per mL. There is no noticeable difference between the two cases: $C_B = C_H$ and $C_H = 0$ for given N_p. In this case, the main source of inoculum effects is the trapping of peptides in bacterial cells. (A) (right) When C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL, the MIC is insensitive to the value of N_p used, as if bacterial cells are in the low-cell density limit (*i.e.*, their presence creates a minimal inoculum effect). At the MIC, host cells are below the MHC. As a result, the binding of peptides to the host-cell membrane is responsible for the slow increase of the MIC with C_H. The presence of a large amount of bacterial cells ($C_B = 10^8$ cells per mL) increases the MIC about ten-fold as long as $C_H \lesssim 10^8$ cells per mL (the two homogenous MIC curves from the graph in the left are also included for comparison purposes.) (B) (left) In all cases, the MHC increases with increasing cell densities: either C_B or C_H. When $C_H = 5 \times 10^9$ cells per mL, the MHC is large and remains roughly flat as C_B increases up to $C_B = 10^9$ cells per mL. It is obviously larger for the larger N_p case (squares or diamonds); it can be two orders of magnitude larger than MHC_0. Also the MHC is larger for the competitive case $C_B = C_H$ compared to the corresponding noncompetitive case $C_B = 0$: at the MHC, the bacterial cells are above the MIC and the resulting peptide trapping in the bacterial cells raises the MHC. (B) (right) The presence of a small concentration of bacteria (*i.e.*, $C_B = 5 \times 10^4$ cells per mL) does not alter the MHC in any significant way. Also the MHC increases faster with C_H for larger N_p, as expected from eqn (4b). In the presence of a large amount of bacterial cells ($C_B = 10^8$ cells per mL), the MHC is about three times as large as in the corresponding host-cell only case, as long as $C_H \lesssim 10^7$ cells per mL.

$= 10^9$ cells per mL. This is consistent with eqn (4b), which suggests that the MHC is roughly independent of C_B, as long as C_H is sufficiently larger than C_B. The MHC is obviously larger for the larger N_p case (squares or diamonds). Finally, the MHC is somewhat larger in the presence of an equal amount of host cells ($C_B = C_H$) compared to the host-cell only case $C_B = 0$. Peptide trapping in the bacterial cells is responsible for this.

As shown in the graph on the right in Fig. 6, the presence of a small concentration of bacteria (*i.e.*, $C_B = 5 \times 10^4$ cells per mL) does not alter the MHC in any significant way. For this, compare

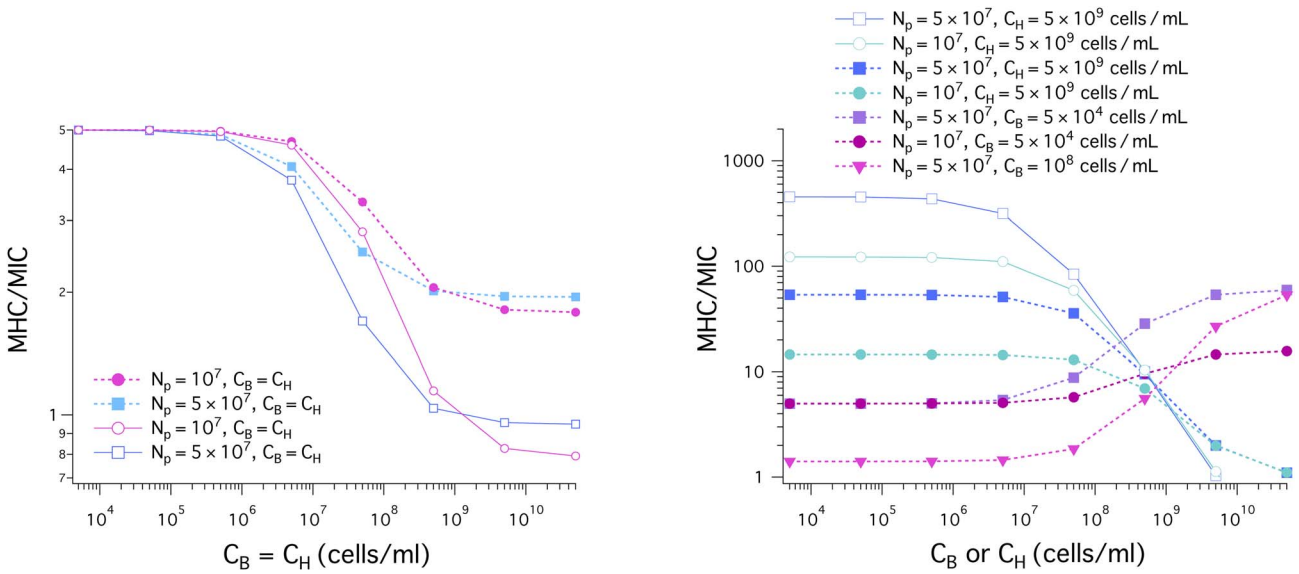

Fig. 7 Cell-density dependence of MHC/MIC for the noncompetitive and competitive cases, represented by solid lines with unfilled symbols and dashed lines with filled symbols, respectively. In the graph on the left, $C_H = C_B$; in the graph on the right, the 'x' axis represents C_B (C_H), when C_H (C_B) is held fixed. We have chosen the same parameters as in Fig. 6: $MIC_0 = 1$ μm and $MHC_0 = 5$ μm; $w_B = -16.6\ k_BT$ and $w_H = -6.72\ k_BT$ as for melittin; the bacterial cell surface area $A_B = 12\ \mu m^2$ (suitable for *E. coli*); the host cell surface area $A_H = 200\ \mu m^2 \approx 17 \times A_B$ (as for human red blood cells); $a_B = 71\ Å^2$ and $a_H = 74\ Å^2$; $v_p = 33^3\ Å^3$ and $A_p = 400\ Å^2$. (left) In all cases shown, $C_H = C_B$. The selectivity, MHC/MIC, decreases as the cell density increases. It is larger for the competitive case (filled symbols), more so for larger $C_H = C_B$. For $C_H = C_B \lesssim 10^9$ cells per mL, the selectivity is somewhat larger when N_p is smaller; in this case, peptide trapping works in bacteria's favor by increasing the MIC. (right) The selectivity obtained with $C_H = 5 \times 10^9$ cells per mL decreases with increasing C_B, more rapidly for larger N_p. In this case, peptide trapping enhances the selectivity as long as $C_B \lesssim 5 \times 10^9$ cells per mL (competitive) or $C_B \lesssim 5 \times 10^8$ cells per mL (noncompetitive) but does not seem to have a noticeable impact outside this range. In contrast, it increases with C_H, more rapidly for larger N_p, when C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL or $C_B = 10^8$ cells per mL. The selectivity is smaller for the latter choice of C_B. With the parameter choices used, the noncompetitive selectivity can be an order of magnitude larger than the corresponding competitive one; depending on how the selectivity is measured, it can be two or three order of magnitude different; for this, compare the blue solid line with open squares with the magenta dashed curve with inverted filled triangles.

open diamonds and filled squares or between open inverted triangles and filled circles. Similarly to the other cases shown on the left in Fig. 6(B), the MHC increases faster with C_H for larger N_p, as expected from eqn (4b). The presence of a large amount of bacterial cells ($C_B = 10^8$ cells per mL) increases the MHC about three-fold from the corresponding host-cell only case, long as $C_H \lesssim 10^7$ cells per mL. For this, compare the dashed curve with filled circles in cyan with open diamonds in blue.

Fig. 7 shows the results for MHC/MIC. The dashed lines with filled symbols represent competitive selectivity, whereas the solid lines with unfilled symbols describe noncompetitive selectivity; in the latter case, MHCs and MICs, obtained for host-cell only and bacteria-only solutions, respectively, are combined into MHC/MIC.

In the graph on the left in Fig. 7, we have chosen $C_H = C_B$. In all cases shown in the graph, the selectivity, MHC/MIC, decreases from the initial value MHC_0/MIC_0 as the cell density increases. It is larger for the competitive case (filled symbols) than for the corresponding noncompetitive case so that the difference between the two cases is more pronounced for larger $C_H = C_B$. For $C_H = C_B \lesssim 10^9$ cells per mL, the selectivity is somewhat larger when N_p is smaller; in this case, peptide trapping works in bacteria's favor by increasing the MIC.

As shown in the graph on the right in Fig. 7, the selectivity obtained with $C_H = 5 \times 10^9$ cells per mL decreases with increasing C_B, more rapidly when N_p is larger. In this case, peptide trapping enhances the selectivity for $C_B \lesssim 5 \times 10^9$ cells per mL (competitive) or $C_B \lesssim 5 \times 10^8$ cells per mL (noncompetitive) but does not seem to have a noticeable impact outside this range, as it approaches MHC_0/MIC_0. In contrast, it increases with C_H, more so for larger N_p, when C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL or $C_B = 5 \times 10^8$ cells per mL. The selectivity is smaller for the latter choice of C_B. The presence of host cells in the competitive case works in favor of the host cells by enhancing the selectivity, more effectively for larger N_p.

The results in Fig. 7 show how the selectivity can be overestimated. With the parameter choices used, the noncompetitive selectivity can be an order of magnitude larger than the corresponding competitive one. Furthermore, depending on how the selectivity is measured, it can be two or three order of magnitude different; for this, compare the solid line with unfilled squares in blue (noncompetitive) and the dashed line with filled squares in purple (competitive).

The picture offered by the graph on the right in Fig. 7 is not only consistent with the earlier observation that the selectivity can be excessively overestimated[11] (see ref. 16 for a theoretical basis) but also clarifies further how peptide selectivity is influenced by various factors or even the way it is measured: competitive, noncompetitive, the presence of host cells, peptide trapping in dead cells.

4.3 Membrane *versus* cell selectivity

There are both similarities and differences between membrane selectivity (Fig. 5) and cell selectivity (Fig. 7) of antimicrobial peptides. In both cases, the membrane-density or cell-density dependence of the selectivity is well manifested. If we set $C_H = C_B$, both membrane and cell selectivity decrease with $C_H = C_B$. In the presence of 5×10^9 cells per mL of host cells or neutral membranes (mimicking host cell membranes) as in whole blood, the selectivity decreases as C_B increases. In both cases, the selectivity tends to be overestimated in a noncompetitive environment with reference to the corresponding competitive case; when $A_H = A_B$, however, the difference between competitive and noncompetitive selectivity against model membranes appears to be minor, especially when $C_B \lesssim 10^9$ cells per mL (Fig. 5). When C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL, the membrane selectivity remains nearly flat as a function of C_H, whereas the cell selectivity increases up to about 10 folds for $N_p = 5 \times 10^7$; if $N_p = 0$, the selectivity would remain nearly flat (the data not shown).

It is worth noting that the MICs for bacterial membranes in Fig. 5 are much smaller than those for bacterial cells in Fig. 6. In contrast, the MHCs in the two figures are comparable. In the case of *E. coli*, the outer membrane enclosing the cell tends to raise MIC_0. In addition, peptide trapping in dead cells is also responsible for the differences between membranes and cells. Nevertheless, the qualitative picture offered from membranes (Fig. 5) is generally consistent with the one obtained for cells.

5. Discussions and conclusions

We have presented a biophysical model of peptide activity and selectivity by combining a pedagogical approach with a Langmuir-type model. If the former captures the cell-density dependence of peptide activity and selectivity in an intuitively-obvious way, the latter relates peptide binding (or trapping) to an effective binding (or trapping) energy.

Using the model, we have clarified how the presence of host cells and peptide trapping influence peptide selectivity and how competitive selectivity differs from noncompetitive selectivity. If the competitive selectivity represents a mixture of bacteria and host cells, the noncompetitive one is obtained by combining MICs and MHCs for bacterium-only and host-cell-only solutions, respectively. In this work, we chose parameters relevant for the peptide melittin (see refs. [19–21] and relevant references therein).

The results based on the model suggest a rather nontrivial dependence of the selectivity on the presence of host cells, cell density, and peptide trapping; these factors or effects can enhance or reduce the selectivity depending on how the density of host cells and that of bacterial cells are chosen. When $C_B = C_H$, the selectivity is somewhat smaller for larger N_p, unless $C_B = C_H$ is sufficiently large (left graph in Fig. 7). In more general cases (right graph in Fig. 7), however, peptide trapping tends to enhance the selectivity; also the presence of host cells works in favor of the host cells, but it raises the MIC up to about 10-fold (Fig. 6(A)).

When $C_B = C_H$, the selectivity decreases from the initial value MHC_0/MIC_0, with increasing $C_B = C_H$, more rapidly for the noncompetitive case; the selectivity is higher for the competitive case and is not sensitive to the choice of N_p. In the presence of a large amount of host cells ($C_H = 5 \times 10^9$ cells per mL), the selectivity decreases with increasing C_B in both competitive and noncompetitive cases. The noncompetitive selectivity can be one-order of magnitude larger than the corresponding competitive one. When C_B is held fixed at $C_B = 5 \times 10^4$ cells per mL or at $C_B = 10^8$ cells per mL, the competitive selectivity increases with C_H; the selectivity is smaller for the latter choice of C_B. Depending on how cell density is chosen, the selectivity can be overly overestimated – almost by three orders of magnitude.

Our work also clarifies how the cell selectivity of AMPs differs from their membrane selectivity. The selectivity based on model membranes is typically larger than the one measured for cells. In both cases (membranes and cells), noncompetitive selectivity is typically larger than the corresponding competitive one, except for the case $C_B = C_H$.

The results in this work suggest that the selectivity reflects not only peptide-membrane parameters but also cell density, peptide trapping, and even the way the selectivity is measured (competitive *vs.* noncompetitive). This is a natural consequence of MICs and MHCs that vary with cell density and N_p. Mapping out possible scenarios of peptide activity and selectivity thus would involve exploring wide ranges of C_B and C_H, which are not easily realized in experiments.

If the involved peptide-membrane parameters are characterized, our model described by eqn (4) can be used as a predictive model. It enables one to calculate MICs, MHCs, and MHC/MIC, as a function of cell density: C_B or C_H, the density of bacterial and host cells, respectively.

Alternatively, eqn (3) can be used as a fitting model for analyzing MIC and MHC data obtained in a noncompetitive manner: the '*y*'-intercept and the 'slope' can be extracted by fitting MIC or MHC data to eqn (3a) or (3b), respectively. This enables one to determine MIC_0 or MHC_0. Eqn (12) shows how these quantities are related to peptide's binding energy w (w_B or w_H) and $P/L^*((P/L)_B^*$ or $(P/L)_H^*)$. It is worth noting that P/L^* has been measured for various model membranes[19–21] as well as for cells.[10] Once P/L^* is known, MIC_0 and MHC_0 can be converted into w_B and w_H, respectively. Conversely, if w is known, P/L^* can be estimated. If all this information is used in the 'slope,' the value of N_p can be extracted.

The information from the homogeneous analysis above can be used in eqn (4), which represents a competitive case. Accordingly, one can quantify peptide selectivity for a biologically relevant setting, which reflects the degree and location of infection. For instance, C_B ranges from 1 colony-forming unit (CFU mL^{-1}) (in blood stream, where $C_H \approx 5 \times 10^9$ cells per mL) to 10^9 CFU mL^{-1} (in soft tissue or peritonea) (see a recent review[12] and relevant references therein).

To advance our model and to take fuller advantage of its predictive power, computational and experimental methods can be employed to evaluate further the respective roles of host cells, cell density, and peptide trapping in the selectivity of

AMPs (see Fig. 6). Because of their complexity, peptide-cell systems are not so amenable to microscopic computational modeling based on molecular dynamics simulations.[32] A concerted effort between theoretical modeling, computational approaches, and experiments would be desired. Along the line of what was done in recent studies,[14] in which a number of key parameters including N_p were extracted, parameters for multi-species cultures can be mapped out and used in eqn (4) or its variation.

In this work and in a typical experimental setting, the total number of AMPs is treated as a constant. In reality, however, it is influenced by the expression of AMPs by the host[14] and peptide degradation by protease.[12,22] Furthermore, earlier studies highlight the stochastic nature of eliminating bacteria with AMPs and its impact on the survivability of a population.[14] It was shown that below the MIC, two sub-populations emerged: one group that stopped dividing and another group that could grow unharmed and divide. To clarify the roles of these population fluctuations, stochastic modeling of population dynamics can be employed.[33,34]

Conflicts of interest

There are no conflicts of interest to declare.

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