Ionic Hydrogen Bonds and Lipid Packing Defects Determine the Binding Orientation and Insertion Depth of RecA on Multicomponent Lipid Bilayers

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Supporting Information

ABSTRACT: We describe a computational and experimental approach for probing the binding properties of the RecA protein at the surface of anionic membranes. Fluorescence measurements indicate that RecA behaves differently when bound to phosphatidylglycerol (PG)- and cardiolipin (CL)-containing liposomes. We use a multistage computational protocol that integrates an implicit membrane/solvent model, the highly mobile mimetic membrane model, and the full atomistic membrane model to study how different anionic lipids perturb RecA binding to the membrane. With anionic lipids studied here, the binding interface involves three key regions: the N-terminal helix, the DNA binding loop L2, and the M-M7 region. The nature of binding involves both electrostatic interactions between cationic protein residues and lipid polar/charged groups and insertion of hydrophobic residues. The L2 loop contributes more to membrane insertion than the N-terminal helix. More subtle aspects of RecA−membrane interaction are influenced by specific properties of anionic lipids. Ionic hydrogen bonds between the carboxylate group in phosphatidylserine and several lysine residues in the C-terminal region of RecA stabilize the parallel (∥) binding orientation, which is not locally stable on PG- and CL-containing membranes despite similarity in the overall charge density. Lipid packing defects, which are more prevalent in the presence of conical lipids, are observed to enhance the insertion depth of hydrophobic motifs. The computational finding that RecA binds in a similar orientation to PG- and CL-containing membranes is consistent with the fact that PG alone is sufficient to induce RecA polar localization, although CL might be more effective because of its tighter binding to RecA. The different fluorescence behaviors of RecA upon binding to PG- and CL-containing liposomes is likely due to the different structures and flexibility of the C-terminal region of RecA when it binds to different anionic phospholipids.

1. INTRODUCTION

Reversible binding of peripheral proteins to cell membrane is involved in many cell signaling and trafficking events.1−12 For example, PKC-C2 (protein kinase C conserved 2) domains are found in proteins such as synaptotagmin 1, which triggers membrane fusion and neurotransmission upon binding to membrane and Ca2+.9 Coverage of membrane by the Bin amphiphsin Rvs (BAR) domains is involved in synaptic vesicle endocytosis, muscle T cell tubule formation, and membrane remodeling.13−17 To understand the function and regulatory mechanism of these and other peripheral proteins, it is important to identify factors that dictate their binding orientation, depth, and affinity to cell membranes. This is not easy to accomplish using experiments alone. Indeed, it has been established that both electrostatic interactions between protein and lipids and insertion of hydrophobic protein motifs into the bilayer contribute to binding,18−21 moreover, protein conformational changes (even unfolding22) and local lipid reorganizations,23 especially when multivalent lipids such as phosphatidylinositol 4,5-bisphosphate (PIP2)24,25 and cardiolipin (CL)26 are present, are often involved. Although several experimental techniques are available for measuring the orientation and insertion depth of specific protein motifs (for example, parallax analysis,27 electron paramagnetic resonance (EPR),28,29 X-ray reflectivity analysis,30 and two-dimensional sum-frequency-generation),31 it is important to supplement experimental studies with computational analyses to obtain a more complete understanding of membrane-binding mechanisms.

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RecA is a DNA repair protein that plays a central role in homologous recombination and mediates the bacterial SOS response. A recent study found that RecA fused to the green fluorescent protein (RecA-GFP) localized to polar regions in Escherichia coli cells. Subsequent studies demonstrated that the cellular localization of RecA overlapped with the distribution of CL at the cell pole. Although lipids have been suggested to be relevant to the SOS response of RecA, little mechanistic detail is available concerning the interaction between lipid membranes and RecA and its biological consequence. In our latest study, we probed RecA-membrane interaction using a combined experimental and computational approach. By measuring RecA binding affinity to liposomes consisting of different lipids and monitoring RecA localization in different strains of bacteria (e.g., those without phosphatidylglycerol (PG)/CL in their membrane), we established that anionic lipids such as PG and CL were essential to the localization of RecA to the cell poles and the initiation of the SOS response; the binding affinity of RecA to PG/CL was in the micromolar range, and significantly lower than the nanomolar binding affinity of RecA to single-stranded DNA. Although membrane binding had little impact on ATP binding to RecA, this interaction decreased the hydrolysis rate of ATP by RecA and was hypothesized to stabilize the filament form of RecA. We proposed that the membrane acts as a scaffold for nucleating the formation of RecA filament bundles and plays an important role in the SOS response.

On the computational side, taking advantage of a free energy method in the framework of an implicit membrane/solvent model (GBSW-GCS), we identified the binding interface of RecA with the surface of membranes consisting of anionic lipids (Figure 1A,B). The semiquantitative methodology also enabled us to predict mutations that substantially reduced the binding of RecA to membranes. Subsequent in vitro and in vivo experiments confirmed that those mutations led to a substantial decrease of RecA binding to anionic membranes and also a significant reduction in polar localization of RecA in E. coli cells. These studies indicate that there is the need for better understanding the interaction between RecA and lipid membranes. For example, in vivo experiments established the importance of anionic lipids; however, it was not possible to clearly distinguish the contributions from PG and CL; because PG is the cellular precursor of CL, it was not possible to remove PG only (but not CL) from the E. coli membrane. Our long-term goal is to understand how the binding of RecA to membrane depends on the identity of the anionic lipid and the biological consequence of such dependence. In this work, we experimentally demonstrate that the behavior of RecA at the surface of a liposome varies when PG is replaced by CL. Next, we focus on computational analysis of RecA binding to lipid bilayers that contain different types of anionic lipids (Figure 2). In particular, we investigate how anionic lipid headgroup and spontaneous curvature impact the binding orientation and clearly distinguish the contributions from PG and CL; because PG is the cellular precursor of CL, it was not possible to remove PG only (but not CL) from the E. coli membrane. Our long-term goal is to understand how the binding of RecA to membrane depends on the identity of the anionic lipid and the biological consequence of such dependence. In this work, we experimentally demonstrate that the behavior of RecA at the surface of a liposome varies when PG is replaced by CL. Next, we focus on computational analysis of RecA binding to lipid bilayers that contain different types of anionic lipids (Figure 2). In particular, we investigate how anionic lipid headgroup and spontaneous curvature impact the binding orientation and.
insertion depth of RecA. The computational results are discussed in the context of available experimental data.

Considering the relatively large size of RecA (333 residues per monomer), we expect that directly simulating its binding interaction with a multicomponent lipid bilayer using a full atomistic model will encounter serious convergence problems; reorientation of a medium-size protein at the membrane surface and reorganization of the nearby lipids is a slow process and therefore difficult to sample adequately using a full atomistic model. Therefore, we advocate a multistage protocol: We start by using an implicit membrane/solvent model (GBSW-GCS) to thoroughly sample the binding orientation of the protein at an anionic membrane surface. Next, the preferred orientations are studied using the highly mobile membrane mimetic (HMMM) model, which treats the lipid headgroups with full atomistic detail but replaces the lipid tail region with organic solvent to accelerate lipid lateral diffusion. Finally, with the local lipid distribution properly equilibrated, the bilayer is restored to full atomistic resolution by replacing the HMMM organic solvent with the realistic lipid tails. In addition to accelerating sampling, our computational protocol incrementally includes more physicochemical details in the protein–lipid interaction. The GBSW-GCS model includes the effect of electrostatic interactions at a mean-field level and also a minimal model for hydrophobic insertion; the HMMM model describes the interactions between protein and lipids with chemical details but does not properly account for effects associated with the spontaneous curvature of lipids, the description of which requires a full atomistic model for the lipid tails. Comparing results from the three types of simulations provides insight into the relative importance of generic electrostatics, chemically specific interactions, and effects associated with lipid tail packing for determining the binding properties of RecA at a lipid membrane surface.

In this paper, we first summarize the computational methods and experimental procedures. These are followed by presentation of results and discussion of various contributions to the binding between RecA and a lipid membrane. We conclude with a few specific remarks concerning RecA–membrane interactions and a brief outlook of future directions.

2. COMPUTATIONAL AND EXPERIMENTAL METHODS

2.1. Computational Methods. As mentioned above, we start from implicit membrane/solvent model (GBSW-GCS) predictions and then refine the results with explicit lipid/solvent models. Specifically, HMMM is used to first equilibrate the local lipid distribution upon RecA binding, then full lipid tails are recovered, leading to the full atomistic membrane simulations. The GBSW-GCS calculations were presented in ref 37, and we include them here for completeness and as a reference for the explicit lipid/solvent simulations.

2.1.1. Implicit Membrane/Solvent Model: GBSW-GCS. In GBSW-GCS, the generalized Born with a simple switching function (GBSW) model is used to describe the dielectric properties associated with the membrane–water interface; the Gouy–Chapman–Stern (GCS) model is used to describe contributions from the anionic lipids and counterions. The GBSW model has been successfully applied to several problems involving transmembrane proteins and the Gouy–Chapman model has been found useful for modeling protein binding to anionic membranes. In our recent work, we emphasized the importance of including the Stern contribution, which may substantially modify the apparent surface charge for a multicomponent lipid bilayer that contains anionic lipids. The key equations and parameters in the GBSW-GCS model are summarized in ref 39; we note here only that the Stern contribution modifies the electrostatic potential at the membrane–water interface

$$\phi(0) = \frac{2}{\beta \varepsilon} \arcsinh \frac{\sigma(1 - x)}{\sqrt{8 \epsilon \rho / \beta}}$$

where $\sigma$ is the unmodified surface charge density that corresponds to the molar fraction of the anionic lipid in the bilayer, $\epsilon$ the elementary charge, $\epsilon$ the absolute permittivity of water, $\rho$ the number concentration of the counterion of valence $Z_c$, and $\beta$ the inverse temperature $1/k_B T$. The charge scaling factor $(1 - x)$ is provided by the solution to Stern’s equation:

$$\frac{1}{K_c} \frac{x}{1 - x} + \frac{2\sigma}{\sqrt{8 \epsilon \rho / \beta}} \left( \frac{x(1 - x)}{K_c \varepsilon} \right)^1 = 1 = 0$$

where $K_c$ is the association constant of the counterions on the membrane surface and $x$ is the molar concentration of the salt: $x = \rho/1000 N_A$. In this study, we use $1 \text{ M}^{-1}$ as the association constant, which was determined for Na$^+$ absorption on a mixed PC–PS bilayer. For a bilayer with 27% anionic lipid, which is the composition used in our study and approximates the concentration in E. coli membranes, the Stern contribution scales down the surface charge density by 62.2%.

Although the GBSW-GCS model is not expected to be quantitatively accurate because of the various approximations inherent to a dielectric continuum model, it is expected to be qualitatively valuable for generating favorable protein orientations at a membrane surface without deep penetration into the bilayer; the implicit solvation framework is also uniquely suited for computing binding free energy following a thermodynamic perturbation framework, which is computationally more efficient than the more commonly used umbrella sampling route. These features were taken advantage of in our previous study of RecA–membrane binding, in which GBSW-GCS was used to predict the protein–membrane binding interface and mutations that reduce binding. Multiple independent GBSW-GCS simulations of length 10–20 ns each were carried out, and these converged to two locally stable conformations, which are referred to as the parallel (∥) and perpendicular (∥) orientations (Figure 1; see below for details).

2.1.2. Explicit Membrane/Solvent Models: HMMM and All-Atom Simulations. Lipid (de)mixing on a membrane patch with ~100 nm$^2$ size occurs on the time scale of microseconds. To enhance lipid (de)mixing, two possibilities are the HMMM model and the semigrand canonical ensemble sampling approach. In a bilayer that has both 2-tailed lipids such as dioleoylphosphatidylcholine (DOPC) and 4-tailed lipids such as tetraoleoylcardiolipin (TOCL), the semigrand canonical ensemble approach is complex to implement. Therefore, we have adopted the HMMM model to equilibrate lipid distribution upon RecA binding. In the HMMM model, the majority of the lipid tail is replaced with an organic solvent, 1,2-dichloroethane (DCE). For example, DOPC (18:1) is replaced by DHPC (6:0) with a layer of DCE liquid. As a result, lateral diffusion of lipids is greatly enhanced; in the current study, we observe an enhancement of ~20-fold, similar to the original report of Takhorshid and co-workers. Previous studies confirmed that amino acid insertion PMFs in HMMM models generally resemble the results from full lipid simulations. Nevertheless, we occasionally observed that...
one or two lipid molecules dissociate spontaneously from the bilayer during equilibration; whenever this happens, the floating lipids are deleted to avoid any impact on protein binding. Fortunately, this happens very rarely in our simulations and the number of lipids deleted is very small ($2-4$ out of 400 lipids).

For each predicted binding orientation of RecA (|| or \perp, see Figure 1A, B), we conduct HMMM simulations for several lipid compositions: 80% DOPC + 20% DOPG (referred to as HMMM-PG20), 80% DOPC + 20% DOPS (HMMM-PS20), and 90% DOPC + 10% TOCL (HMMM-CL10). We take the protonation state of CL such that each CL is featured with a negative charge of $-2$. In addition to PG and CL, PS is also chosen because it is a prevalent anionic lipid in many cell membranes. A technical point concerns the HMMM-CL10 simulations: due to the significant spontaneous curvature of CL, they cluster to bulge out of the bilayer plane, leading to substantial structural instability and hole formation in the HMMM bilayer. Therefore, in HMMM-CL10 simulations, the CL headgroups were harmonically restrained to remain in the same \textit{z} plane as the rest of PC head groups; specifically, a harmonic restraint with a force constant of 0.2 kcal mol$^{-1}$Å$^{-2}$ is applied to all P atoms of CL. The system size of HMMM simulations is generally $110 \times 110 \times 110$ Å$^3$. As shown in Figure S1, the number of lipid molecules near RecA (within a 10 Å radius) indeed changes fairly rapidly during the HMMM simulations for all three mixed lipid bilayers; this is not the case with the mixed-lipid simulations. Due to the spontaneous curvature of CL, the 100% CL bilayer remains structurally stable in all setups, including those with PG20, PS20, or CL10, following 100 ns of equilibration before performing energy minimization and MD equilibration.

For each RecA–membrane setup (|| or \perp binding orientation with PG20, PS20, or CL10), following 100 ns of equilibration using the HMMM model, the full lipid tails are recovered. To do so, the organic solvent DCLE molecules are deleted and the tails of the corresponding lipids are regrown. An in-house python code and CHARMM c37a1 are used to process such "mutations", although a similar approach is available at the CHARMM-GUI Web site. Strong steric contacts are removed before performing energy minimization and MD equilibration. Thereafter, the system is simulated for at least 150 ns to probe RecA orientation and interaction with nearby lipids. The bilayer is free of any structural restraints and remains structurally stable in all setups, including those with CL; the latter observation is consistent with the experimental result that a bilayer can tolerate up to 30% of TOCL.

As elaborated later in section 3.3, to probe the impact of lipid packing defect on RecA binding, we also conduct several simulations with a small fraction of dioleoylglycerol (DOG): 80% DOPC + 10% DOPS + 10% DOG (referred to as PSDOG simulation) and 80% DOPC + 10% DOPG + 10% DOG (PGDOG). DOG is a conical shaped lipid that has been shown to introduce packing defects in membranes. The simulation protocol is generally similar, i.e., 100 ns of HMMM simulations are followed by at least 150 ns of full atomistic membrane simulations. Because DOG is charge neutral and therefore may not colocalize with RecA, a weak wall restraining potential is applied to 2 PS/PG and 1 DOG at a lateral distance of 15 Å from the N-terminal helix, and on 4 PS/PG and 2 DOG surrounding the Loop L2.

Finally, two sets of simulations with pure anionic lipids (DOPS and TOCL, respectively) are also carried out for comparison with the mixed-lipid simulations. Due to the relatively homogeneous nature of these bilayers, no HMMM equilibration step is used. Despite the large negative spontaneous curvature of CL, the 100% CL bilayer remains stable due likely to the use of the periodic boundary condition; the simulation can be regarded as a model for a local CL-enriched domain at the cell pole. The results for these simulations are summarized in the Supporting Information.

The starting structure of RecA in the GBSW-GCS simulations is taken from the crystal structure 3CMT, which contains a RecA oligomer bound to a dsDNA; a monomer structure is taken, and the nucleotide is also removed from the ATP binding site because no nucleotide was added in the fluorescence experiments. In all simulations, the protein is described with the charmm27 force field with CMAP corrections. The lipid parameters are taken from charmm36 force field with NBFIX for ionic–carbonyl interactions; CL force fields are generously provided by the Pastor group, and DOG force field parameters are generated with the CGenFF Web site. Bonds involving hydrogen are constrained with SHAKE to enable a 2 fs time step. The Lennard-Jones potential cutoff is set to be 14 Å with a switching function turned on at 10 Å. Electrostatics are treated with particle-mesh Ewald with a grid size of 1 Å. NPAT (isobaric–isothermal ensemble) with constant area on x–y plane is adopted for HMMM models to ensure stable bilayers. For the full membrane simulations (after the tails are recovered), NPT (isobaric–isothermal ensemble with targeted surface tension) is adopted for production runs, where surface tension is set to 0 dyn/cm.

All explicit membrane simulations are carried out with the NAMD 2.9 package. 2.1.3. Additional Analysis: Headgroup Packing Defect Estimation. Head group packing defects are often discussed in the context of protein–membrane interactions.

In principle, another interesting approach for characterizing protein insertion into the bilayer is to compute the effect of binding on the lateral pressure profile in the membrane and resulting membrane curvature. Such calculations, however, appear to converge slowly, especially in the presence of the protein at the membrane surface; therefore, we have not pursued curvature calculations extensively.

2.2. Experimental Methods. We measured changes in the fluorescence intensity of Trp290 and 309 (see Figure 3B) during the titration of RecA with liposomes. We fixed the excitation of a PTI Quantamaster 300 spectrofluorimeter (Birmingham, NJ) at $\lambda$ = 295 and collected fluorescence data at 37 °C. We titrated liposomes consisting of 100% (weight) PC (negative control), 100% PG (weight), and 80:20% PC:CL (weight) into purified \textit{E. coli} RecA (0.7 μM) in 1.4 mL of R buffer (20 mM Tris-HCl, 0.1 mM EDTA, pH 7.4) and mixed the suspension thoroughly using a pipet. We incubated mixtures for 5 min at 37 °C and imaged the fluorescence emission by scanning $\lambda$ = 300–400 nm. We corrected measurements of fluorescence intensity for the dilution of RecA during titration. We also accounted for scattering of light by liposomes and corrected our fluorescence measurements by subtracting values of fluorescence that we measured for liposome-only titrations into buffer.
different anionic lipids modulate RecA–membrane interactions using computational studies. For example, previous GBSW-GCS simulations predicted two favorable binding orientations (Figure 1) in which the Trp residues are in very different distances from the membrane–water interface. Therefore, it is possible that different anionic lipids favor specific binding orientations.

3.2. The Binding Interface Predicted by GBSW-GCS is Supported by Explicit Membrane Simulations. In the two predicted binding orientations of RecA, the binding interfaces are largely consistent and involve the N-terminal helix (1−21: AIDENKQKALAAALGQIEKQF) and the DNA binding loop, L2 (195−205: IRMKIIVMFGN). With the GBSW-GCS model, the \( \parallel \) orientation is more stable than the \( \perp \) orientation by \( \sim 4 \) kcal/mol, because the C-terminal region (which has several conserved lysine residues) is engaged in additional electrostatic interactions with the anionic bilayer. For each of these two binding orientations, we have conducted explicit membrane simulations with different lipid compositions (PS20, PG20, and CL10) and different lipid models (full atomistic versus HMMM). Generally speaking, the explicit membrane simulations support that the two binding orientations remain the most relevant ones, although the identity of the anionic lipid and the lipid model have notable impacts on the stability of these binding orientations. In this subsection, we comment on the consistent aspects from different implicit and explicit membrane simulations to highlight the most salient nature of RecA–membrane interaction; in the next subsection, we analyze in detail the variations among different simulations to gain insights into factors that dictate more subtle aspects of RecA binding to membrane surface.

In Figure 4 (panels A and B for PS20, D and E for PG20, and G and H for CL10), we show representative snapshots for the two binding orientations from explicit membrane simulations; also shown are the average \( z \) position of RecA residues relative to the membrane surface (the value of 0 indicates the average position of phosphorus atoms in the upper leaflet; panel C for PS20, F for PG20, and I for CL10). Comparison between those and the results from the GBSW-GCS simulations (Figure 1) indicates that the protein–membrane interface predicted from the implicit membrane/solvent model remains largely unchanged when explicit chemical details are introduced to describe membrane–protein interactions. Indeed, the general protein–membrane interaction interface still involves the N-terminal helix and the DNA binding loop L2, although the N-terminal helix appears to interact less with the membrane that contains PS as the anionic lipids (see next subsection); these observations are consistent with the fact that predicted mutations in those regions were experimentally confirmed to lead to weaker binding to membranes that contain PG or CL and substantially less localization of RecA to the bacterial cell pole.

A few other regions are also not too far from the membrane surface. For example, loop L1 (157−165, GEMGDISHMG) is generally close to the membrane surface according to the insertion profiles in Figures 1C and 4C,F,I and residue M164 dips into the membrane in the CL10-L simulation; however, because the sequence of L1 does not feature any cationic residues or hydrophobic residues, its location relative to the surface is likely a simple consequence of its spatial proximity to L2, and L1 is not expected to contribute much to binding affinity. Another region close to the membrane surface is the M-M7 region, which is a key interface between RecA
monomers when assembled into filaments; the sequence corresponds to 247–257 (VKNKIAAPFKQ), which clearly features both cationic and hydrophobic residues. The M-M7 region therefore contributes to binding in both implicit and explicit membrane simulations, although its proximity is more evident in the explicit membrane simulations than in GBSW-GCS simulations (compare Figures 1C and 4C,F,I). Compared to the N-terminal helix and L2 loop, however, residues in M-M7 region do not insert as deeply; thus, the interaction with the membrane is largely electrostatic in nature rather than hydrophobic insertion.

In short, the most fundamental physical factors that dictate RecA–membrane interaction include (i) electrostatic attraction between the cationic residues in several key regions (N-
terminal helix, the L2 loop and M-M7) and anionic lipids and (ii) insertion of hydrophobic residues in the L2 region into the bilayer.

3.3. Variations among Different Simulations: Factors That Dictate Protein Binding Orientation and Insertion Depth. 3.3.1. Binding Orientation: Importance of Ionic Hydrogen Bonds to Headgroup–Protein Interactions. To describe more subtle differences in the binding orientations from different simulations, we monitor two properties: (i) the distance between the center of mass of the pair of Trp residues (Trp290, Trp 309) to the phosphorus atoms of the upper membrane leaflet and (ii) the number of hydrogen bonding interactions between the C-terminal residues (260–310, including backbone atoms) and lipid polar/charged groups (including glycerol, carboxylate, phosphate, and NH$_3^+$). The cutoff distance for a hydrogen bonding interaction is taken to be 3 Å between the potential donor and acceptor atoms, and no angular criterion is imposed. The results are shown in Figures 5 and 6, respectively (for the time-dependence of the Trp–membrane distance, see Figure S2). The two properties appear to be highly correlated: when the Trp–membrane distances are larger than 25 Å, the average number of hydrogen bonds between the C-terminal region and lipids is essentially 0, indicative of a \( \perp \) orientation; when the Trp–membrane distances are less than 25 Å, there are many C-terminal–lipid hydrogen bonds, indicative of a \( \parallel \) orientation. We note that the M-M7 region also features hydrogen bonds with the membrane (see Figure S3), although the differences among different simulations are less striking and therefore not considered for distinguishing the two binding orientations.

As shown in Figure 5A, the most consistent trend among the full atomistic membrane simulations is that the \( \perp \) orientation remains locally stable for all three membranes (PS20, PG20, and CL10); the average Trp–membrane distances are all around 45 Å, only slightly lower than the value of \( \sim 52 \) Å from GBSW-GCS simulations. This similarity is unlikely due to limited sampling, because the full atomistic membrane simulations start from equilibrated HMMM configurations, which in fact feature fairly different Trp–membrane distances for the \( \perp \) orientation (see Figure 5B), i.e., there is sufficient time during the 150–200 ns of atomistic simulations for RecA to adjust its orientation on the membrane. This is further illustrated by the observation that the \( \parallel \) orientation is less stable than the \( \perp \) orientation on some membrane surfaces.

Compared to the GBSW-GCS \( \parallel \) orientation, the Trp–membrane distances from the explicit membrane simulations are notably longer and the stability of the \( \parallel \) orientation depends critically on the identity of the anionic lipid. In the PS20–\( \parallel \) simulation, the average Trp–membrane distance is \( \sim 23 \) Å (Figure 5A), and there is a large number of ionic hydrogen bonds between the C-terminal residues and lipids (Figure 6A); these involve Lys residues in the C-terminal region (Lys 280, Lys 282, and Lys 286) interacting with either the phosphate group from PC (Figure 7A) or with the carboxylate group in PS (Figure 7B). In the PG20–\( \parallel \) simulation, the average Trp–membrane distance is substantially larger and approaches 30 Å (Figure 5A; also see Figure S2 for the time dependence of Trp–membrane distance); this is correlated with hydrogen bonding interaction between the C-terminal lysine residues and the PG phosphate group (Figure 7C) forming only transiently during the MD simulation (Figure 6B). In the CL10–\( \parallel \) simulation, RecA quickly reorients and converts to the \( \perp \) orientation, leading to even longer Trp–membrane distances (\( \sim 35 \) Å, Figure 5A) and a negligible number of hydrogen bonds (Figure 6C) between the C-terminal lysine residues and the TOCL phosphate group (Figure 7D). Apparently, the carboxylate group in PS plays an essential role in stabilizing the \( \parallel \) orientation of RecA; this is further supported by the observation that the \( \perp \) orientation spontaneously converts to the \( \parallel \) orientation on a pure PS bilayer while the \( \parallel \) orientation still evolves toward the \( \perp \) orientation on a pure CL bilayer surface (Figure S4).
Another curious difference among the full atomistic membrane simulations is that the N-terminal helix (residue 1–21) binds to the membrane in both PG20 and CL10 simulations, but it detaches from the membrane in both || and ∥ PS20 simulations (see Figure 4). The detachment of the N-terminal helix from PS surface is reproducible in independent simulations, including in a simulation that contains 100% PS (see the Supporting Information).

Compared to full atomistic membrane simulations, HMMM simulations are not always in agreement in terms of the orientation of RecA. For example, for both PG- and CL-containing membrane simulations, the HMMM model leads to substantially shorter Trp–membrane distances for both || and ∥ trajectories (compare panels A and B of Figure 5). In

HMMPG20, a large local deformation of the bilayer is observed upon RecA binding, leading to shorter distances between C-terminal residues and lipids. In HMMML10, as mentioned above, the CL headgroups were restrained in the z direction to help maintain the structural stability of the membrane; this clearly affects the membrane–protein interaction, leading RecA to adopt an orientation intermediate between || and ∥ in both sets of trajectories that started from || and ∥, respectively. These differences between HMMM and full atomistic membrane simulations suggest that lipid packing, which controls hydrophobic insertion of protein motifs into the bilayer and is not properly described in HMMM, may also play a role in determining the binding properties of the protein at the membrane surface. In the next subsection, we analyze this aspect of RecA–membrane interactions.

3.3.2. Insertion Depth: Importance of Lipid Packing Defects. As shown in the insertion profiles in Figure 4, two key regions insert into the bilayer: the N-terminal helix and the DNA binding loop L2; the only exception is that the N-terminal helix detaches from the membrane surface in the PS20 simulations. We examine how membrane composition perturbs the insertion depth of these motifs; in particular, we summarize the insertion depth (defined by the center of mass position of the side chain relative to the average phosphorus positions) of two hydrophobic residues, Ile2 and Phe203 in Table 1.

The DNA binding loop L2 (195–205: IRMKIGVGMFG) features several hydrophobic residues, and in most explicit membrane simulations, the insertion depth is fairly comparable, e.g., Phe203 is about 4–5 Å below the phosphorus plane. The only apparent exception is that the insertion is shallower (∼1 Å below the phosphorus plane) for the || orientation on the PS20 membrane surface. For the N-terminal helix (1–21: AIDENKQKALAAALGQIEKQF), there appears to be more variations. In || simulations, the N-terminal helix develops a break around Ala12 in the GBSW-GCS simulations, and the break remains stable throughout both HMMM and full atomistic membrane simulations. While a kink in the helix has been observed to maximize hydrophobic insertion of antimicrobial peptides,89 whether the helical break develops is not strongly correlated with the binding orientation of RecA; for example, both sets of CL10 simulations converge to the || orientation although the helical break is inherited from the GSBW-GCS simulations. 

There appears to be a weak correlation between insertion depth and the helical conformation. As shown in Table 1, the insertion of I2 is notably deeper with the ∥ set of trajectories for both PG20 and CL10, which feature an unbroken helix.

Table 1. Location of I2 and F203 Relative to the Membrane Phosphorus Plane (Measured in Angstroms) in Different Explicit Membrane Simulations

<table>
<thead>
<tr>
<th>simulation</th>
<th>I2</th>
<th>F203</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>traj</td>
<td></td>
</tr>
<tr>
<td>PS20</td>
<td>41.0 (10.8)</td>
<td>31.2 (4.7)</td>
</tr>
<tr>
<td>PG20</td>
<td>7.4 (1.4)</td>
<td>−3.0 (1.5)</td>
</tr>
<tr>
<td>CL10</td>
<td>2.1 (2.1)</td>
<td>−3.5 (2.4)</td>
</tr>
<tr>
<td>PSDOG</td>
<td>−0.2 (1.9)</td>
<td>−</td>
</tr>
<tr>
<td>PGDOG</td>
<td>−0.7 (1.6)</td>
<td>−</td>
</tr>
</tbody>
</table>

“Defined by the center of mass position of the side chain relative to the average phosphorus positions in the upper leaflet. Values in parentheses are the standard deviations. “Notation for different simulations: PS20, 80% DOPC + 20% DOPS; PG20, 80% DOPC + 20% DOPG; CL10, 90% DOPC + 10% TOCL; PSDOG, 80% DOPC + 10% DOPS + 10% DOG; PGDOG, 80% DOPC + 10% DOPG + 10% DOG. “Throughout this work, “traj || (∥)” indicates the MD simulation that starts with a || (∥) binding orientation predicted by GBSW-GCS calculation.
Because both CL10 simulations (which started with parallel and perpendicular orientations) converge to the perpendicular orientation, and in both cases the N-terminal helix and L2 loop generally insert fairly deeply into the bilayer, one hypothesis is that deep insertion of these protein motifs is correlated with the perpendicular orientation. To further test this hypothesis, we have conducted simulations that mix the conical shaped DOG into PC/PS and PC/PG bilayers. The expectation is that DOG further enhances packing defects and therefore deeper insertion of hydrophobic residues, which in turn may favor the perpendicular binding orientation of RecA and destabilize the parallel binding orientation as observed in CL10 simulations. As shown in Table 2, introducing DOG indeed substantially increases the packing defect in the bilayer; in fact, the percentiles of packing defect in DOG-containing bilayers are even higher than that in CL10 simulations (compare panels A and B of Figure 8 with panels C and D). As a result, both the N-terminal helix and the L2 loop insert notably deeper into the DOG-containing bilayers (see Table 1 for I2/F203 insertion and Figure 9C,D for more complete insertion profiles); indeed, in PSDOG simulations, although the N-terminal helix started from an unbound conformation (as observed in the PS20 simulations, see Figure 4C), it ends up at an insertion depth comparable to PGDOG. The deeper insertions, however, do not lead to a change in the binding orientation of RecA from parallel to perpendicular on the PSDOG membrane surface, as evidenced by the Trp−membrane distances and hydrogen-bonding statistics for the C-terminal−membrane interactions (Figure 9A−B); these properties of RecA in PGDOG simulations are generally similar to those in the PG simulations as well. Therefore, the relative stability of the parallel and perpendicular orientation is more directly modulated by the ionic hydrogen bonds between the C-terminal region of RecA and lipid head groups, and insertion depth of key protein motifs plays little, if any, role in this context. As shown in the Supporting Information, this is further supported by the pure PS and CL simulations, which showed that the perpendicular orientation converted spontaneously to parallel in the pure PS simulation, while this does not occur in the pure CL simulations because of the lack of carboxylate in the lipid headgroup.

3.4. Structural Difference between RecA on Different Membranes. By comparing the pairwise RMSDs of RecA in different environments (Figure 10A), we find that RMSDs of RecA on PG20 membrane are the smallest compared to the crystal structure (3CMT), in which RecA oligomerizes and is bound to a dsDNA. The largest RMSDs are observed from CL10 and PS20 simulations. In PS20 simulations, the N-terminal helix does not bind to the membrane (Figure 4) but remains a stable α-helix in solution; the C-terminal region is thus twisted to avoid steric contacts with the N-terminal helix. In CL10 simulations, the converged perpendicular binding mode leads to high fluctuations in certain regions; specifically, a short β-sheet region in the C-terminal (G265 to Y271) unfolds to a random coil with large fluctuations (compare secondary structure analysis in Figure 10A and three-dimensional structures in Figure 10D,E).

Because the starting structure in our simulations is obtained from a crystal structure in which RecA forms an oligomer and binds to a dsDNA, it is not too surprising that the protein structure deviates from the starting structure during explicit membrane simulations, and some of the structural differences among different membrane simulations are likely stochastic in nature. Nevertheless, it is interesting that the protein maintains its structure in close agreement with the dsDNA bound form at the surface of PG20 layer, regardless of the initial binding orientation. This seems to be consistent with the importance of PG to the function of RecA in bacteria. The origin for the substantial structural difference between RecA in PG20 and CL10 simulations is not immediately clear. This difference, however, might contribute to the different fluorescence behaviors of the pair of Trp residues observed experimentally when RecA binds to liposomes that contain PG and CL (Figure 3, see Discussion).

3.5. Discussion. 3.5.1. Factors That Dictate RecA Binding Orientation and Insertion Depth. The computational studies are motivated by the observation that fluorescence from the pair of Trp residues behaves differently when RecA binds to PG- or CL-containing liposomes (Figure 3); the fluorescence intensity increases as RecA binds to CL-containing liposomes, while the opposite trend is observed with PG. The hypothesis we aim to test in this computational study is that the binding orientation and insertion depth of RecA at the membrane...
surface is dependent on the identity of the anionic lipids, leading to different microscopic environments of the Trp residues and therefore distinct fluorescence spectra.

The extensive set of simulations we describe indicates that the binding orientation of RecA is similar in the PG20 and CL10 systems; in both cases, RecA adopts a binding orientation that features no persistent hydrogen bonding interaction between the C-terminal domain and the membrane, regardless of the initial binding orientation (Figure 6). The distance between the pair of Trp residues and the membrane is shorter in the PG20-|| simulations (~30 vs ~40 Å for CL10-||), see Figure 5), but the general binding interface is very consistent in the PG20 and CL10 simulations and implicates the N-terminal helix, the DNA binding loop L2, and the M-M7 region in this protein–membrane interface (see Figure 4). The insertion depths of these key motifs, especially L2, appear to be deeper in CL10 than in PG20 (see Table 1) because of the packing defect induced by the large negative spontaneous curvature of CL (Table 2 and Figure 8); the magnitude of the difference, however, is rather modest when there is only 10% CL in the membrane.

These relatively subtle differences between the PG20 and CL10 systems do not support the original hypothesis that PG and CL lead to significantly different binding orientations of RecA at the membrane surface; thus, the observed difference in Trp fluorescence arises from other factors, such as water exposure, residue conformations, and the local electric field. The fact that the fluorescence peak position is very similar for CL- and PG-binding suggests that the environments of the Trp residues are not drastically different. Indeed, we have computed the solvent accessible surface area (SASA) for the Trp residues in different sets of trajectories and found that the results are largely similar (Table S1). Therefore, the change of fluorescence intensity is more likely to come from more subtle differences between the Trp residues when RecA binds to different membranes. For example, we observe that one of the Trp residues adopts different orientations with respect to the membrane normal when RecA binds to PG or CL membranes (Table S1); thus, the electric field sensed by this Trp residue is expected to be different, and this may lead to different quantum yields for the fluorescence process. The molecular dynamics simulations also indicate that the protein conformation and flexibility in the C-terminal region of RecA differ when it is bound to PG- and CL-containing membranes (Figure 10). Without detailed fluorescence calculations, however, it remains difficult to determine the origin of the intensity difference; conducting detailed QM/MM dynamics analysis along this line would be very informative.

PG and CL have similar head groups (CL is synthesized in the cell with PG as the precursor), and in hindsight, it is not surprising that they interact with protein motifs in generally similar ways. By contrast, PS features a carboxylate group, which is observed to form stable ionic hydrogen bonds with the cationic groups in the C-terminal region of RecA. As a result, the || orientation predicted from the implicit membrane/solvent model (GBSW-GCS) remains most stable in the PS20 simulation (Figure 5A and Figure 6A); in fact, on a pure PS bilayer surface, the || orientation is observed to spontaneously convert to the ⊥ orientation to form ionic hydrogen bonds (Figure S4). Charge density alone is not the key factor because the ⊥ orientation remains favored at a pure CL bilayer surface (Figure S4), which features the same charge density as the pure PS bilayer but without the carboxylate groups. The importance
of carboxylate group in PS might be one of the reasons behind its prominence in cell signaling. Hydrophobic insertion generally contributes to the binding of peptides and peripheral proteins.\(^{39,85,89}\) In the case of RecA, insertion of loop L2 (which contains both cationic and hydrophobic residues) appears most important because it is observed in all explicit membrane simulations. The N-terminal helix inserts more shallowly in some cases and curiously detaches from the PS surface (Figure 4 and Figure S5). Simulations with added conical lipids (DOG) and pure CL lipids indicate that lipid packing defects favor deeper insertion, although the magnitude of the effect is large only when there is a large number of CL molecules (see Figure S5 for CL100 results). Therefore, packing defect effects are likely to be most prominent for protein binding to a microdomain consisting of lipids with a high spontaneous curvature. HMMM simulations leading to somewhat different binding orientations compared to full atomistic membrane simulations (panel A versus B in Figure S5) suggest that lipid packing effects can not be ignored for studying membrane–protein association. For RecA, packing defects affect primarily the insertion depth (thus likely binding affinity) and play a less important role than ionic hydrogen bonds in determining the binding orientation.

3.5.2. Roles of Different Lipids and Relation to RecA Localization in Cells. One of the long-term goals for our study is to understand the roles of lipids in cellular localization of proteins and the impact on protein structure and function. In the case of RecA, our previous study\(^{37}\) found that anionic lipids are crucial to its polar localization. Removing CL from the bacterial membrane did not abolish localization of RecA, suggesting that PG may be sufficient for localizing RecA to the cell pole. This is not contradictory to the experimental observation\(^{34}\) that RecA polar localization overlaps with CL polar localization; the higher binding affinity of RecA to CL than to PG\(^{37}\) implies that CL is more effective at recruiting RecA to the polar cell regions. The effectiveness of PG for recruiting RecA is consistent with the computational results here, which point to a generally similar binding orientation of RecA to PG- and CL-containing membranes; we have not yet estimated the relative binding affinity of RecA to PG versus CL, although the deeper insertion of RecA in CL10/CL100 simulations compared to PG is consistent with tighter binding to a CL enriched membrane. Invoking only PG for the polar localization of RecA requires an understanding of the driving force for PG to localize to the cell poles. In contrast to the large spontaneous curvature of CL, PG has a small spontaneous curvature and therefore is not expected to localize to the cell pole based on elastic energy alone.\(^{66,67}\) In this context, it is worth mentioning that the radius of curvature at the bacterial cell pole is at the scale of micrometers, much larger than the radius of spontaneous curvature of individual lipids (for CL, it is \(\sim 1 \text{ nm}^{66,67}\)); thus, cellular localization of lipids might involve multiple driving forces that are electrostatic\(^{56}\) and elastic\(^{56}\) in nature, respectively. Therefore, factors that drive cellular localization of lipids need to be better understood.

PS appears to drive a different binding orientation of RecA, which is intriguing and should be verified experimentally; whether altering the binding orientation leads to a significant impact on the assembly of RecA filaments and downstream events is also an important unknown question. In the E. coli cells studied in our work, the amount of PS in the membrane is minimal (<1% of total phospholipids) and therefore not relevant. In other cell membranes, however, PS is abundant.\(^{39,52}\) The interaction between PS and RecA (and its homologues\(^{91}\)) in regulating DNA repair activity is a topic worth exploring.

Our previous work\(^{37}\) focused on the involvement of the N-terminal helix and the L2 loop in RecA binding to membrane. The current work further highlights the importance of electrostatic interactions due to the M-M7 region, and several lysine residues in the C-terminal domain (Lys 280, 282, and 286) for the \(|\parallel|\) binding orientation. These residues are candidates for future mutation experiments that further explore key regions of RecA that dictate the membrane binding activity.

Finally, we emphasize that our computational studies have so far focused on the interaction between a single RecA with the membrane. To fully understand how membrane binding is related to RecA filament formation and competition with DNA binding,\(^{52}\) it is clearly important to study the much larger system that includes RecA oligomers, DNA, and membrane. This will be pursued in the near future.

4. CONCLUDING REMARKS

As a continuation of our recent work,\(^{37}\) we use a combined experimental and computational approach to analyze RecA interaction with the surface of anionic lipid membranes. Fluorescence spectroscopy measurements clearly indicate that
RecA behaves differently when bound to PG- and CL-containing liposomes. Using computational studies, we probed how lipid composition perturbs RecA binding properties to membranes, such as the binding orientation and insertion depth.

By comparing results from extensive molecular dynamics simulations that use anionic lipids with different head groups (PG/CL versus PS) and different spontaneous curvatures (PG/PS versus CL, DOG), we gained insights into factors that dictate the binding properties of RecA at anionic membrane surfaces. For all the anionic lipids studied here, the binding interface involves three key regions: the N-terminal helix, the DNA binding loop L2, and the M-M7 region; the nature of binding involves both electrostatic interactions between cationic protein residues and lipid polar/charged groups and insertion of hydrophobic residues. The L2 loop contributes more to membrane insertion than the N-terminal helix.

The physicochemical properties of anionic lipids influence subtle aspects of RecA–membrane interaction. Ionic hydrogen bonds between the carboxylate group in PS and cationic protein residues in the C-terminal region of RecA stabilize the binding orientation, which is not locally stable on PG- and CL-containing membranes despite similarity in the overall charge density. Lipid packing defects, which are more prevalent in the presence of conical lipids (e.g., CL, DOG), are observed to enhance the insertion depth of hydrophobic motifs. For RecA, the magnitude of the effect is modest unless a large number of conical lipids are present; thus, the binding orientation is more sensitive to ionic hydrogen bonds between the protein and lipids compared to insertion depth.

From a technical point of view, our study also highlights the advantage of integrating GBSW-GCS, HMMM, and full atomistic membrane-based simulations for probing protein binding to the surface of multicomponent membranes. First, the three-stage protocol helps efficiently sample both protein binding orientation and local lipid distributions; for a relatively large protein such as RecA, directly sampling binding with full atomistic details is likely to encounter serious sampling issues. Second, by comparing results from different types of simulations that feature different levels of physicochemical details of protein–lipid interactions, novel insights can be obtained regarding factors that dictate specific aspects of binding. For example, the binding interface is largely consistent among the different models and suggests that generic electrostatic interactions involving cationic protein residues and anionic lipids are dominant elements. The binding orientation is favored in the GBSW-GCS model but not in most explicit membrane simulations (except for PS), which underscores the roles of specific chemical interaction between the protein and lipid groups (e.g., with carboxylate in PS). Finally, the difference between HMMM and full atomistic membrane simulations highlights that lipid packing effects can not be ignored when studying membrane–protein interactions.

In the context of building connections to experimental observations, the computational results do not support different binding orientations as the reason for the different fluorescence behaviors of RecA upon binding to PG- and CL-containing liposomes; rather, the difference is likely due to the different structures and flexibility of the C-terminal region when RecA binds to the surface of different anionic membranes. The computational result demonstrating that RecA binds in a similar orientation to PG- and CL-containing membranes is consistent with the observation that PG is sufficient to induce RecA polar localization in E. coli cells, although CL might be more effective because of its tighter binding to RecA. Key questions that require further joint experimental and computational studies include the mechanism of cellular localization of different lipids\textsuperscript{66,95} (e.g., PG), verification of the binding orientation in PS-containing membrane, and potential connections between binding orientation and filament formation of RecA; going beyond RecA and probing cytoplasmic proteins’ interaction with membrane at a systems level is also an exciting direction. Clearly, the path of understanding how phospholipids regulate the cellular localization of peripheral membrane proteins and their downstream processes is beginning to emerge.

### ASSOCIATED CONTENT

#### Supporting information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b02164.

Additional results and analysis of lipid distributions in HMMM simulations and several sets (PS20, PG20, CL10, PS100, and CL100) of simulations (PDF)

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**Notes**

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