# Binding of realistically-shaped peptides to membrane curvature

**Anisotropic membrane curvature sensing by antibacterial peptides**
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*Recommended with a commentary by Peter Olmsted, Georgetown University.*

Lipid bilayer membranes are deceptively complex. They comprise two leaflets composed of a complex mixture of lipids and proteins, and were originally treated as featureless 2D fluids [1]. For decades they were thought to act only as flexible walls enclosing the cell, the nucleus, and other internal structures necessary for cell function. With the discovery of nanodomains with elevated levels of cholesterol and/or saturated lipids ('rafts') [2] there has been a realization that the constituents of bilayers (lipids*, cholesterol, membrane-bound proteins) play a crucial role in regulating membrane function: membranes form the core of the protein synthesis and delivery mechanisms in the sponge-like Golgi Apparatus, control the transport of materials within cells, regulate ion and pH content between different environments, and regulate binding and recognition between the cell and proteins, peptides, viruses, other cells, and nucleic acids. Interaction at and across membranes is integral to immune response, the formation of spherical membrane vesicles to enter or exit cells, and numerous other processes. An attractive hypothesis is that the many components of the membrane permit multiple and even parallel responses to stimuli, perhaps mediated by an underlying critical point [3].

In a recent ArXiv paper, Gómez-Llobregat *et al.* study the important topic of protein recognition by membranes, in terms of the coupling between protein shape and membrane curvature which can lead to curvature sensing. The free energy per unit area of a bent membrane is usually assumed to take the form derived by Helfrich [4]:

$$G = \frac{1}{2}\kappa(C_1 + C_2 - C_0)^2 + \bar{\kappa}C_1C_2, \tag{1}$$

where $\kappa$ is the (mean) curvature modulus, $\bar{\kappa}$ penalizes the Gaussian curvature $C_1C_2$, and $C_1, C_2$ are the membrane's two principal curvatures. The spontaneous curvature $C_0$ vanishes for symmetric membranes, as in most *in vitro* experiments.

Lipid shape influences the spontaneous curvature of a leaflet*. Conical lipids with large or charged heads induce inward curvature towards the tail group; inverse-conical lipids with small heads induced outward curvature, and 'flat' lipids induce negligible curvature. Since almost all biological membranes separate different chemical environments and comprise leaflets with different lipid compositions, $C_0$ should be non-zero by symmetry, but is often small (perhaps because the environmental and leaflet asymmetries nearly cancel).

Living membranes contain roughly 18-70% embedded proteins, by mass. Proteins have even more complex shapes than lipids, and perturb the membrane to induce local curvature

* Lipids typically have a polar head group and two tail groups that are short hydrocarbon chains, sometimes with one or more double bonds ('unsaturated')

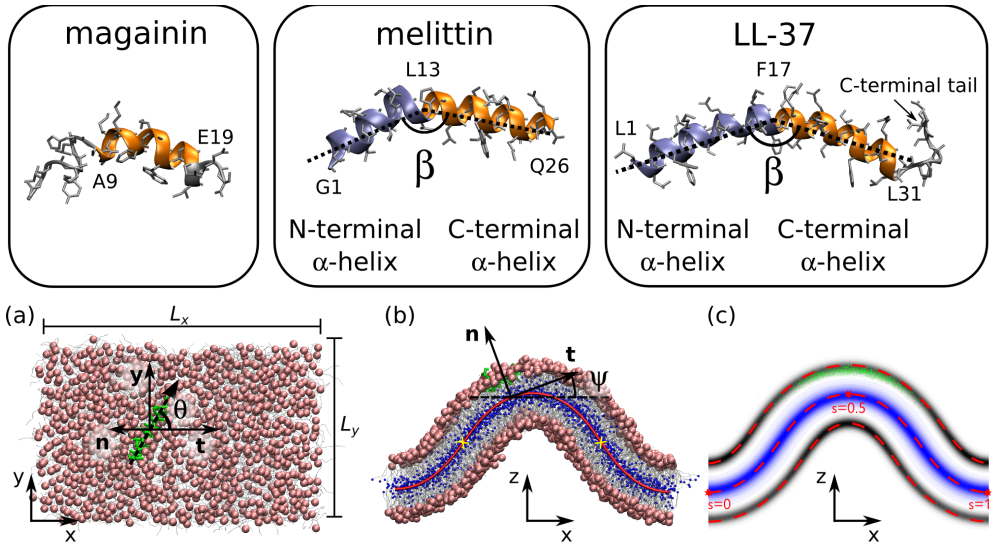


FIG. 1. (top) Three peptides used by Gómez-Llobregat *et al.*; (bottom) Buckled membrane showing a peptide lying in the plane at an angle $\theta$ with respect to the buckling wavevector $\hat{\mathbf{t}}$.

or enforce a local membrane shape. This perturbation leads to membrane-mediated protein-protein interactions similar to the Casimir effect[†] [5], which can influence protein mixing, aggregation, and function [6]. Despite work on the effects of proteins *in* the membrane, surprisingly little attention has been paid to the detailed anisotropic interaction between a protein of a given shape and different local membrane curvatures. An example is membrane *curvature sensing*, whereby protein binding depends on curvature and thus couples protein and membrane functions via curvature [7].

Gómez-Llobregat *et al.* study curvature sensing in a detailed set of simulations. They prepare a model (symmetric) membrane from a mixture of unsaturated lipids with similar head groups. The bilayer is forced to buckle by area compression, which introduces a straight wrinkle, like a wide breaking ocean wave, into the membrane. Such a wave has curvature $C_\perp$ that varies in only one dimension (Fig. 1). Because the curvature parallel to the wavecrest $C_\parallel$ vanishes, the Gaussian curvature $C_\perp C_\parallel$ is zero. Different peptides are equilibrated in the membrane, and their affinities for different curvatures and peptide orientation are extracted from long molecular dynamics simulations [‡]. The peptides have different structures: (magainin) a single $\alpha$-helix; or two $\alpha$-helices linearly linked with a flexible (melittin) or stiff (LL-37) kink angles. These simple peptides display distinctly different alignment properties within the membrane due to the overall non-symmetric nature of the molecules.

Through an elegant set of algorithms the probability distribution $\rho(C, \theta)$ can be extracted from the simulations (Fig. 2). Interestingly, the stiffer peptide sits at two preferred alignment angles that are not related by symmetry to the degree of curvature. That is, the peptide doesn't simply tilt 'right' for one sign of curvature and equally 'left' for another sign of curvature. The peptide's intrinsic shape lacks symmetry and interacts in a non-trivial manner with the *three*-dimensional structure of the membrane. This complexity is probably

[†] Membrane bending fluctuations play the role that vacuum fluctuations play in the standard Casimir effect.

[‡] The lipids and proteins were modeled using the MARTINI force-field [8], which allows significant coarse-graining while retaining many features of the constituent molecules

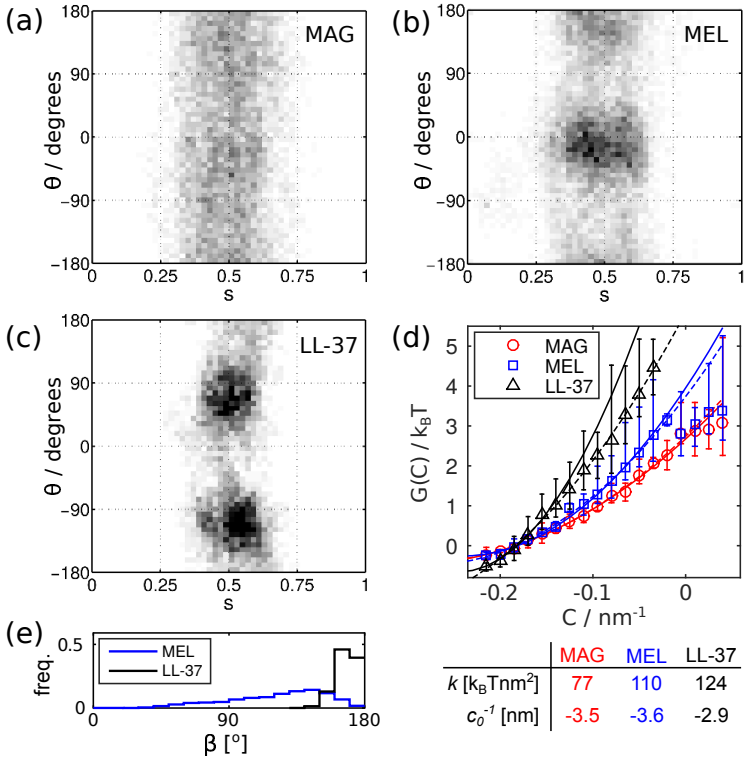


FIG. 2. (abc) Probability distributions $\rho(s(C), \theta)$ for the three peptides. The arc-length position $s$ along the buckled membrane is directly related to the local curvature $C$. (d) Inferred orientationally-averaged free energy of binding $G(C)$ for the three proteins. (e) Distribution of the inter-$\alpha$-helix kink angle $\beta$ for the two larger peptides, showing the stiffness of peptide LL-37. The table shows a fit to an orientationally averaged binding energy $G_b = \frac{1}{2}k(C - C_0)^2$.

general for stiff proteins and peptides, or any complex body embedded in a membrane.

The effective free energy for this distribution shows significant convexity as a function of curvature, which indicates that the binding energy for even such simple peptides is not a simple linear function $G_b \sim C_{ij}H_{ji}$, where $C_{ij}$ is the local (2D) curvature tensor and $H_{ij}$ parametrizes the shape and binding potential of the protein. Hence a quadratic binding potential is necessary and probably generic. The most general quadratic binding potential is $G_b = \frac{1}{2}(C_{ij} - C_{0ij})M_{ijkl}(C_{kl} - C_{0kl})$, where the spontaneous curvature $\overleftrightarrow{C}_0$ characterizes the protein shape, and the symmetric tensor $\overleftrightarrow{M}$ contains up to 6 degrees of freedom.

A simple version of this model by Akabori & Santangelo [9] cannot reproduce the angular dependence of the stiffer peptide LL-37. Gómez-Llobregat *et al.* suggest the variant

$$G_b = \frac{1}{2}K\left(C_1 + C_2 - C_0\right)^2 + b\left(C_1 - C_2\right)\cos\left[2\left(\theta - \alpha\right)\right], \tag{2}$$

which corresponds to rotating the local principal axes of spontaneous curvature by an angle $\alpha$ with respect to the protein axis $\theta$. This does a remarkably good job, and shows that the local curvature directions imposed (and sensed) by the peptide are not trivially related to its local rough symmetry axes, but depend in a complex way on peptide structure and shape.

Note that sensing curvature (and binding) and imposing curvature (bending the membrane) are two sides to the same coin. In these simulations the membrane did not bend due to the adsorbed peptides; larger curvature sensing proteins could *impose* curvature, which can effect changes in membrane shape and potentially induce budding [7, 10].

This work suggests several directions and implications. The simulations could not address coupling to non-zero Gaussian curvature, which may be very important. Distinguishing the binding based on Gaussian curvature could influence the budding of spherical vesicles required for transport within cells, or the branching of membrane structures prevalent in the Golgi and the Endoplasmic Reticulum. The lack of mirror symmetries in proteins implies a non-trivial structure associated with binding, which can influence membrane protein assembly into pores, and potentially select for chirality during translocation. It would be interesting to consider realistic membranes with small amounts of highly curving lipids, to study how lipid compositional fluctuations can interact with curvature-sensing proteins.

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