

A One-Dimensional Continuum Elastic Model for Membrane-Embedded Gramicidin Dimer Dissociation

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Abstract

Membrane elastic properties, which are subject to alteration by compounds such as cholesterol, lipid metabolites and other amphiphiles, as well as pharmaceuticals, can have important effects on membrane proteins. A useful tool for measuring some of these effects is the gramicidin A channels, which are formed by transmembrane dimerization of non-conducting subunits that reside in each bilayer leaflet. The length of the conducting channels is less than the bilayer thickness, meaning that channel formation is associated with a local bilayer deformation. Electrophysiological studies have shown that the dimer becomes increasingly destabilized as the hydrophobic mismatch between the channel and the host bilayer increases. That is, the bilayer imposes a disjoining force on the channel, which grows larger with increasing hydrophobic mismatch. The energetic analysis of the channel-bilayer coupling is usually pursued assuming that each subunit, as well as the subunit-subunit interface, is rigid. Here we relax the latter assumption and explore how the bilayer junction responds to changes in this disjoining force using a simple one-dimensional energetic model, which reproduces key features of the bilayer regulation of gramicidin channel lifetimes.

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Introduction

Membrane protein function can be regulated by changes in membrane lipid composition [1–8]. This regulation may be due to specific binding to the membrane protein or to changes in bilayer collective properties, such as thickness or lipid intrinsic curvature [9]; the collective properties will be the focus of the present analysis. The latter, physical regulation is important because membrane protein properties change when the membrane lipid composition is altered [9], and because many bioactive molecules are amphiphiles that for thermodynamic reasons [10,11] will alter lipid bilayer properties, which may provide insight into why amphiphiles modify the function of numerous different membrane proteins [12,13,14,15], (see [16] for a review.) The diversity of membrane proteins that are regulated by a given amphiphile suggests that these compounds may alter membrane protein function by mechanisms that do not involve direct binding to the target protein. In support of this notion, these amphiphiles alter lipid bilayer properties, as sensed by the bilayer-spanning gramicidin channels, at the concentrations where they are promiscuous modulators of membrane protein function. It thus is likely that changes in continuum membrane properties may, quite generally, regulate the function of bilayer-embedded proteins ranging from receptors over channels to transporters and pumps [9]. This is important because drugs – such as genistein [12], capsaicin [13], curcumin [15] and 2,3-butanedione monoxime [17], that may act through specific binding to their target protein over a given concentration range, alter the function of many different membrane proteins at higher concentrations:

concentrations at which they modify, to varying degrees, the bulk continuum bilayer properties. These changes in bilayer properties can in turn affect the function of disparate membrane proteins [16], which may lead to undesired side effects [18,19].

Many different probes have been used to explore how small molecules alter lipid bilayer properties [20,16]. A particularly useful probe is the gramicidin channel, which is formed from trans-bilayer association of two non-conducting subunits that align “head-to-head” so as to make a continuous, water-filled pore capable of conducting current across the membrane. As such, single-channel measurements can probe the distributions of the monomeric and dimeric states as a function of bulk membrane properties [16]. Specifically, experimental results confirm the hypothesis that the dimer becomes increasingly disfavored with increasing bilayer thickness.

These results usually are interpreted by assuming that the conducting dimer is rigid, compared to the host bilayer, and that there is tight hydrophobic coupling between the membrane-spanning gramicidin dimer and the host bilayer, meaning that the lipid bilayer adjusts to the channel such that the bilayer hydrophobic thickness at the channel-bilayer boundary is equal to the dimer’s hydrophobic length. It is further assumed that the channel dissociation occurs as an all-or-nothing phenomenon – meaning that the subunit-subunit interface is assumed to be rigid (until it breaks). While this description is able to account for the observed changes in single-channel lifetime as a function of the channel-bilayer hydrophobic mismatch [21,16], the subunit-subunit interface is unlikely to be rigid. We therefore expand on the classic description by introducing a simplified one-dimensional

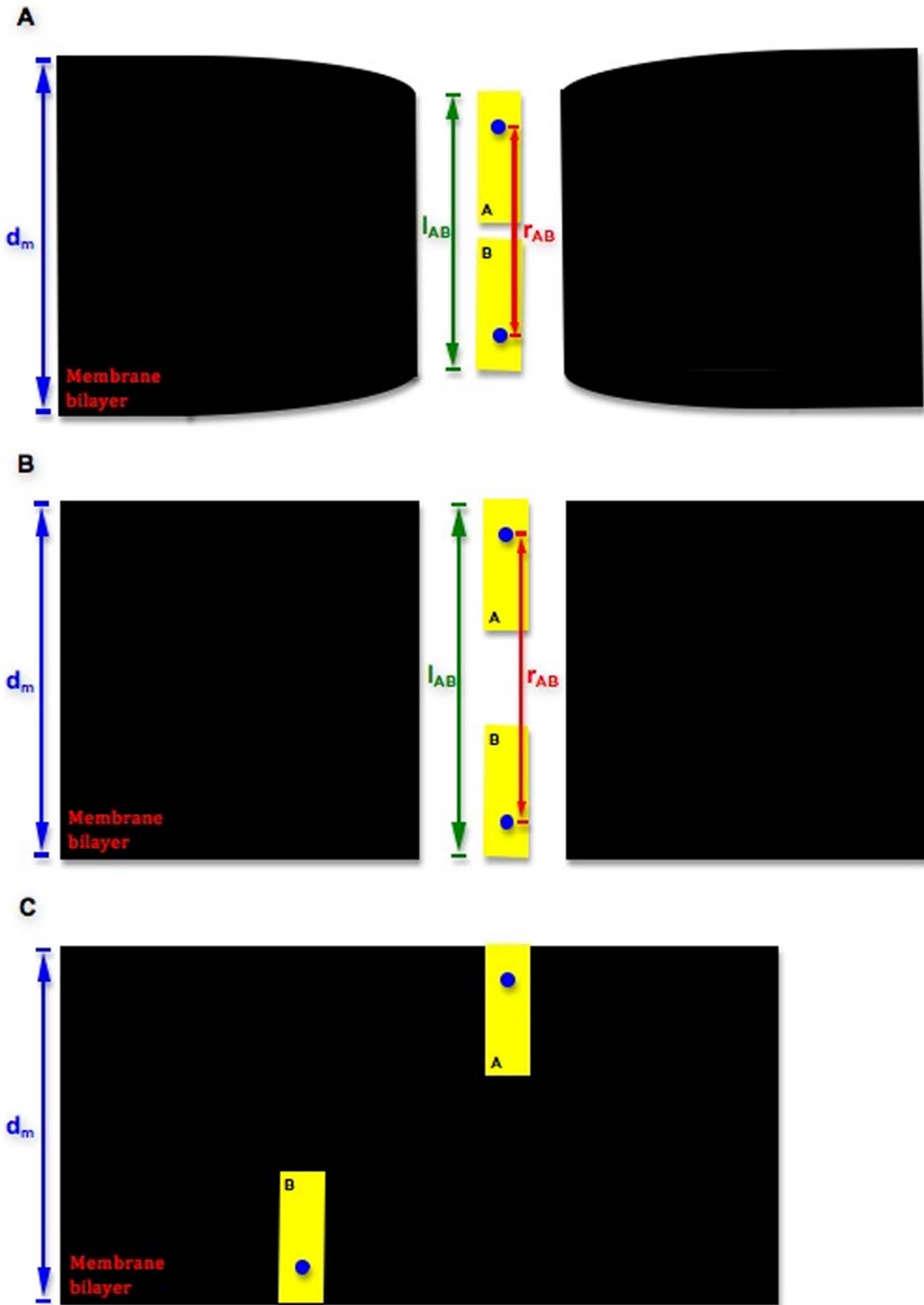


Figure 1. General System Schematic. General schematic of our system. We employ the centers of mass for gramicidin monomers A and B as landmarks to measure monomer separation along the membrane normal axis. The bilayer thickness is denoted by d_m , and l_{AB} is the hydrophobic length of the gramicidin dimer. The hydrophobic mismatch is given by $d_m - l_{AB}$. (a) The dimer-state system with equilibrium inter-monomer separation, $r_{AB} = r_{AB,eq}$. (b) The dissociated state at maximal r_{AB} value. (c) The dissociated state in which monomers A and B are free to move laterally in the plane of the membrane.
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description of the bilayer property-dependent channel dissociation with a flexible monomer-monomer interface. The “tug-of-war” between membrane and dimer becomes evident and the resulting changes in dimer lifetime as a function of changes in bilayer thickness reproduce those known from experiment. When we extend the analysis to changes in bilayer stiffness, we again recover the experimentally observed trends. The model is not quantitatively predictive, as it simplifies a complex three-dimensional and many-body problem to a one-dimensional two-body problem. Yet, it illustrates a key phenomenon at play, namely how increasing bilayer thickness affects the joining force between the two subunits, as the bilayer “pulls” the gramicidin dimer apart, and it shows that introducing flexibility at the subunit-subunit interface does not alter the linear relation between channel-bilayer hydrophobic mismatch and the disjoining force acting to pull the dimer apart.

Methods

Functional form of the potential and experimental parameters

We construct a simple one-dimensional potential to model the gramicidin dimer embedded in a phospholipid bilayer. A general illustration of our simplified system is shown in Figures 1. Figure 1a represents the dimer state at equilibrium. Figure 1b shows the state at which the monomers have separated fully along their common axis. This state gives rise to the dissociated system in Figure 1c, wherein the two monomers have moved apart also in the plane of the bilayer. While the two subunits move apart (or toward each other) axially, the potential consists of a monomer-monomer interaction term $V_M(r_{AB})$ between monomers A and B , and a membrane-dimer interaction term $V_{harm}(r_{AB}; d_m, k_m)$, where r_{AB} is the distance between A and B 's centers of mass, d_m is the membrane thickness and k_m is the membrane stiffness. The monomer-monomer term seeks to restrain A and B so as to keep r_{AB} near its equilibrium value of $r_{AB,eq}$. A Morse potential of this form has long been widely used for two-body interaction energies. The membrane-dimer term consists of a harmonic potential that seeks to relieve the hydrophobic

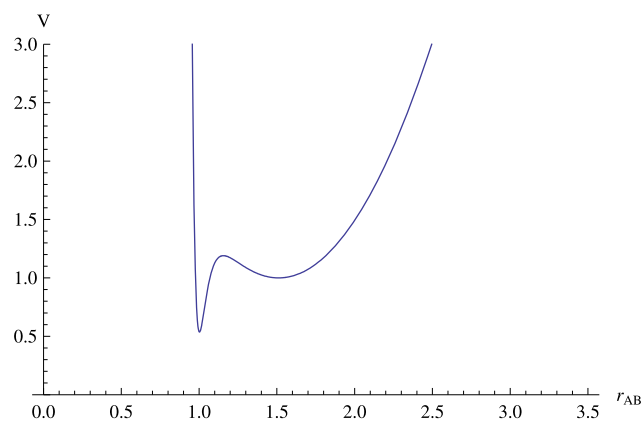


Figure 2. Potential Energy Curve. One-dimensional potential, in units of the dissociation energy D_{AB} , as a function of intermonomer center of mass separation r_{AB} , the latter measured in units of equilibrium distance $r_{AB,eq}$.
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mismatch $d_m - l_{AB} = d_m - (r_{AB} + [l_{AB,eq} - r_{AB,eq}])$ between dimer and membrane, where l_{AB} is the dimer hydrophobic length with equilibrium value $l_{AB,eq}$ [21]. Our values for d_m and $l_{AB,eq}$ are based on the standard reference model for gramicidin embedded within a membrane [22]: $d_m = 2.14 r_{AB,eq}$ and $l_{AB,eq} = 1.63 r_{AB,eq}$.

The full potential is given by

$$V = (V_M(r_{AB}; D_{AB}, \beta_{AB}, r_{AB,eq})) + (V_{harm}(r_{AB}; d_m, k_m, l_{AB,eq}, r_{AB,eq})) \\ = (D_{AB} \{1 - \exp(-\beta_{AB}[r_{AB} - r_{AB,eq}])\}^2) \\ + (k_m \{d_m - (r_{AB} + [l_{AB,eq} - r_{AB,eq}])\}^2), \quad (1)$$

where D_{AB} is the dimer dissociation energy and β_{AB} is the corresponding Morse stiffness parameter for the subunit-subunit interaction.

In the conducting state the two monomers are held together by two to six hydrogen bonds [23,24], though intermediate (presumably low-conductance) states with two and four hydrogen bonds are likely to exist as intermediaries during channel formation and dissociation. The equilibrium distribution of dimers to monomers should be about one to a hundred [25]. We take D_{AB} to be 60 kJ/mol . The equilibrium center of mass separation $r_{AB,eq}$ is 13.3 \AA . We shall measure all energies in units of D_{AB} and all distances in units of $r_{AB,eq}$. At 310K , $k_B T = 0.043 D_{AB}$. From the atomistic energy profile of Miloshevsky and Jordan [24] with membrane dielectric constant equal to one, the Hookean force constant k_{AB} between the monomers can be estimated to be $911.1 D_{AB}/r_{AB,eq}^2$. Then the approximation that near the equilibrium distance,

$$\beta_{AB} \approx \sqrt{\frac{k_{AB}}{2D_{AB}}}, \quad (2)$$

yields $\beta_{AB} = 21.3/r_{AB,eq}$. In the theory of elastic bilayer deformations [22], three bilayer material constants (thickness, and the elastic compression and bending moduli) can be combined into a single phenomenological membrane Hookean force coefficient k_m .

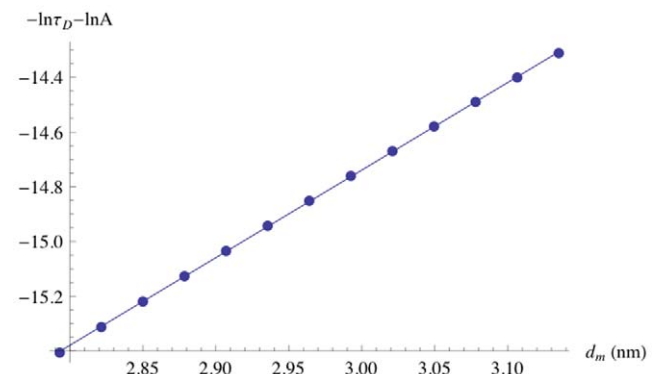


Figure 3. Membrane Thickness Dependence of Dimer Lifetime. The dependence of the dimer lifetime τ_D on membrane thickness d_m , measured in units of nanometers.
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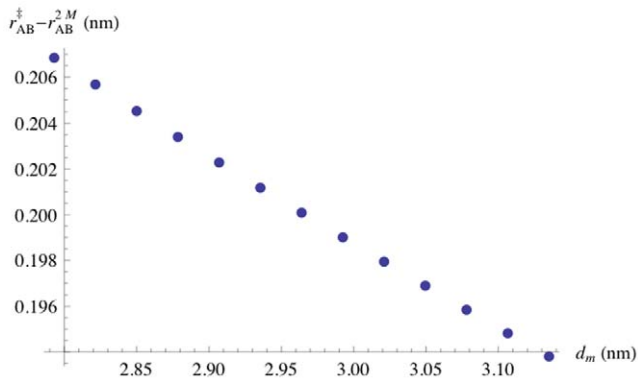


Figure 4. Membrane Thickness Dependence of Distance Between Dimer and Transition State. The distance between dimer and transition state as a function of membrane thickness d_m , with both quantities being measured in units of nanometers. doi:10.1371/journal.pone.0015563.g004

Lundbaek and Andersen [21] estimated k_m to be $2.1D_{AB}/r_{AB,eq}^2$ for the gramicidin dimer embedded in a monoglyceride membrane with no solvent.

Analysis of the potential

Figure 2 shows that Equation (1) with this parameter set yields a double-well potential as a function of r_{AB} . The well on the left (minimum at $r_{AB} = r_{AB}^D$) represents the dimer state of gramicidin – corresponding to Figure 1a – and that on the right (minimum at $r_{AB} = r_{AB}^{2M}$) is the separated two-monomer state – corresponding to Figure 1b. A clearly defined transition state ($r_{AB} = r_{AB}^{\ddagger}$) separates the two wells, and we can apply Transition State Theory [26] to express the rate constant for dissociation. Accordingly, we may extract a rate constant $k_{D \rightarrow 2M}$ for the dissociation process via the Arrhenius equation:

$$k_{D \rightarrow 2M} = A \exp(-E_a/k_B T), \quad (3)$$

where A is a pre-exponential factor (the Arrhenius factor), k_B is the Boltzmann constant, T is temperature and $E_a = V(r_{AB}^{\ddagger}) - V(r_{AB}^D)$ is the activation barrier from the dimer state to the dissociated state. Noting that $k_{D \rightarrow 2M} = \frac{1}{\tau_D}$, where τ_D is the average dimer

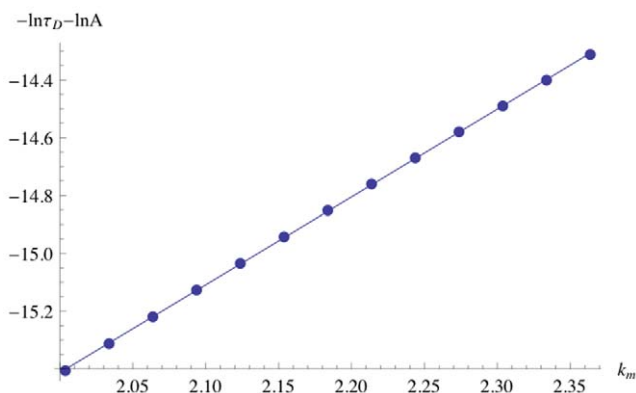


Figure 5. Membrane Stiffness Dependence of Dimer Lifetime. The dependence of the dimer lifetime τ_D on membrane stiffness k_m , measured in units of $D_{AB}/r_{AB,eq}^2$. doi:10.1371/journal.pone.0015563.g005

lifetime, we have

$$-\ln \tau_D - \ln A = -E_a/k_B T. \quad (4)$$

Results

As shown previously [21,16], it is possible to extract the value of a phenomenological spring coefficient from the slope of the relation between $-\ln(\tau)$ and the channel-bilayer hydrophobic mismatch $d_0 - l$, where d_0 is the bilayer hydrophobic thickness and l is the channel hydrophobic length – assuming that the distance from the energy minimum for the conducting dimer to the transition state for dimer dissociation does not vary as a function of the hydrophobic mismatch. Given the potential expressed by Equation (1), the relation between $-\ln \tau_D$ and bilayer thickness remains approximately positive linear (Figure 3), in agreement with experimental results [21,16]. We therefore examined further how changes in bilayer thickness altered the position of the transition state, i.e. how far the two subunits would move apart in order to reach the transition state, where the channels would stop conducting and move away from each other in the plane of the membrane. The results show that the distance the subunits move apart decreases as the bilayer thickness increases, but that the changes are small, as seen in Figure 4.

To examine the experimental trends for varying bilayer stiffness, we scanned through a range of k_m values about the previously noted experimental value while holding membrane thickness d_m constant. The resulting $-\ln \tau_D$ profile, displayed in Figure 5, is an approximately linear relationship similar to that of Figure 3. Though it is not possible to do a quantitative comparison to experimental results, the data reflect the experimental observations [27,12,13,14,15].

Discussion

Gramicidin dimer and membrane are engaged in a “tug of war” in which the dimer “wants” to stay close to its equilibrium separation, while the membrane “wants” to pull the monomers apart so as to relieve the bilayer deformation that is caused by the hydrophobic mismatch. The simple two-well potential curve of Figure 2 illustrates two states: the well ($r_{AB} = r_{AB}^D$) in which the dimer has “won” and stayed in one piece; and the well ($r_{AB} = r_{AB}^{2M}$) in which the membrane has “won” and successfully pulled the monomers apart to match up the channel’s hydrophobic length with the membrane thickness. The actual dissociation is, of course, more complex than depicted here, most likely being a coupled separation along the bilayer normal and rotation/translation in the plan of the bilayer [28]. The key point here is that the monomers separate along their major axes (i.e. r_{AB} increases), then at some point fully break apart, and finally drift apart in the plane of the membrane (Figure 1c).

Subject to this limitation, our model demonstrates that the essential features of the dimer dissociation remain intact as we vary bilayer thickness. Doing so, the distance to the transition state decreases as the bilayer thickness increases (as the disjoining force increases), but the relative change in this distance is modest, indicating that it is indeed possible to use the lifetime-thickness relation, as determined using gramicidin channels, to estimate changes in bilayer properties. A more detailed analysis, with coarse-grained or atomistic simulation, is needed to thoroughly understand the mechanism of gramicidin separation, this process’s dependence on the membrane’s chemistry and bulk properties, and the limitations encountered in the experiments.

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Author Contributions

Conceived and designed the experiments: JNS OA. Performed the experiments: JNS. Analyzed the data: JNS OA. Wrote the paper: JNS OA.

References

- Allen T, Andersen O, Roux B (2006) Ion Permeation through a Narrow Channel: Using Gramicidin to Ascertain All-Atom Molecular Dynamics Potential of Mean Force Methodology and Biomolecular Force Fields. *Biophysical Journal* 90: 3447–3468.
- Huang H (1986) Deformation Free Energy of Bilayer Membrane and its Effect on Gramicidin Channel Lifetime. *Biophysical Journal* 50: 1061–1070.
- Helfrich P, Jakobsson E (1990) Calculation of Deformation Energies and Conformations in Lipid Membranes Containing Gramicidin Channels. *Biophysical Journal* 57: 1075–1084.
- Galbraith T, Wallace B (1998) Phospholipid Chain Length Alters the Equilibrium Between Pore and Channel Forms of Gramicidin. *Faraday Discussions* 57: 159–164.
- Andersen O, et al. *Methods in Molecular Biology*, vol 400: Methods in Membrane Lipids. Totowa, NJ: Humana Press Inc.
- Allen T, Andersen O, Roux B (2004) Energetics of Ion Conduction Through the Gramicidin Channel. *PNAS* 101: 117–122.
- Dzikovski B, Borbat P, Freed J (2004) Spin-Labeled Gramicidin A: Channel Formation and Dissociation. *Biophysical Journal* 87: 3504–3517.
- Andersen O. Kinetics of ion movement mediated by carriers and channels. *Meth Enzymol* 171: 62–112.
- Andersen O, Koeppe R, II (2007) Bilayer Thickness and Membrane Protein Function: an Energetic Perspective. *The Annual Review of Biophysics and Biomolecular Structure* 36: 107–130.
- Evans E, Rawicz W, Hofmann A (1995) Lipid bilayer expansion and mechanical disruption in solutions of water-soluble bile acid. *Dordrecht: Bile Acids in Gastroenterology: Basic and Clinical Advances*, Kluwer Academic Publishers. pp 59–68.
- Zhelev D (1998) Material property characteristics for lipid bilayers containing lysolipid. *Biophys J* 75: 321–30.
- Hwang T, Koeppe R, II, Andersen O (2003) Genistein can modulate channel function by a phosphorylation-independent mechanism: importance of hydrophobic mismatch and bilayer mechanics. *Biochemistry* 42: 13646–13658.
- Lundbaek J, et al. (2005) Capsaicin regulates voltage-dependent sodium channels by altering lipid bilayer elasticity. *Mol Pharmacol* 68: 680–689.
- Bruno M, Koeppe R, II, Andersen O (2007) Docosahexaenoic Acid Alters Bilayer Elastic Properties. *PNAS* 104: 9638–9643.
- Ingolfsson H, Koeppe R, II, Andersen O (2007) Curcumin is a modulator of bilayer material properties. *Biochemistry* 46: 10384–10391.
- Lundbaek J, et al. (2010) Lipid bilayer regulation of membrane protein function: gramicidin channels as molecular force probes. *J R Soc Interface* 7: 373–395.
- Artigas P, et al. (2006) 2,3-Butanedione Monoxime Affects Cystic Fibrosis Transmembrane Conductance Regulator Channel Function through Phosphorylation-Dependent and Phosphorylation-Independent Mechanisms: The Role of Bilayer Material Properties. *Mol Pharmacol* 70: 2015–26.
- Andersen O (2008) Perspectives on how to drug an ion channel. *J Gen Physiol* 131: 395–7.
- Lundbaek J (2008) Lipid bilayer-mediated regulation of ion channel function by amphiphilic drugs. *J Gen Physiol* 131: 421–429.
- Seddon A, et al. (2009) Drug interactions with lipid membranes. *Chem Soc Rev* 38: 2509–19.
- Lundbaek J, Andersen O (1999) Spring Constants for Channel-Induced Lipid Bilayer Deformations Estimates Using Gramicidin Channels. *Biophysical Journal* 76: 889–895.
- Nielsen C, Goulian M, Andersen O (1998) Energetics of Inclusion-Induced Bilayer Deformations. *Biophysical Journal* 74: 1966–1983.
- Busath D, Szabo G (1998) Low Conductance Gramicidin A Channels are Head-to-Head Dimers of $\beta^{6,3}$ -Helices. *Biophysical Journal* 53: 689–695.
- Miloshevsky G, Jordan P (2004) Gating Gramicidin Channels in Lipid Bilayers: Reaction Coordinates and the Mechanism of Dissociation. *Biophysical Journal* 86: 92–104.
- Goulian M, et al. (1998) Gramicidin Channel Kinetics Under Tension. *Biophysical Journal* 74: 328–337.
- Eisenberg D, Crothers D (1979) *Physical Chemistry with Applications to the Life Sciences*. The Benjamin/Cummings Publishing Company, Inc.
- Lundbaek J, et al. (1996) Membrane Stiffness and Channel Function. *Biochemistry* 35: 3825–3830.
- Miloshevsky G, Jordan P (2006) The Open State Gating Mechanism of Gramicidin A Requires Relative Opposed Monomer Rotation and Simultaneous Lateral Displacement. *Structure* 14: 1241–1249.