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# Randomly organized lipids and marginally stable proteins: A coupling of weak interactions to optimize membrane signaling☆

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ABSTRACT

Eukaryotic lipids in a bilayer are dominated by weak cooperative interactions. These interactions impart highly dynamic and pliable properties to the membrane. C2 domain-containing proteins in the membrane also interact weakly and cooperatively giving rise to a high degree of conformational plasticity. We propose that this feature of weak energetics and plasticity shared by lipids and C2 domain-containing proteins enhance a cell's ability to transduce information across the membrane. We explored this hypothesis using information theory to assess the information storage capacity of model and mast cell membranes, as well as differential scanning calorimetry, carboxyfluorescein release assays, and tryptophan fluorescence to assess protein and membrane stability. The distribution of lipids in mast cell membranes encoded 5.6–5.8 bits of information. More information resided in the acyl chains than the head groups and in the inner leaflet of the plasma membrane than the outer leaflet. When the lipid composition and information content of model membranes were varied, the associated C2 domains underwent large changes in stability and denaturation profile. The C2 domain-containing proteins are therefore acutely sensitive to the composition and information content of their associated lipids. Together, these findings suggest that the maximum flow of signaling information through the membrane and into the cell is optimized by the cooperation of near-random distributions of membrane lipids and proteins. This article is part of a Special Issue entitled: Interfacially Active Peptides and Proteins. Guest Editors: William C. Wimley and Kalina Hristova.



## 1. Introduction

A cell membrane is a pliable and responsive surface. Changes in the local environment of a given lipid are propagated in all directions within a leaflet by the weak cooperative interactions between each lipid molecule and its six nearest neighbors [1]. This type of propagation behavior suggests that the membrane has great signaling potential. Another key facet to consider with regard to biological membranes and signaling is the high degree of lipid species diversity. The distribution and diversity of lipids in eukaryotic membranes are thought to maintain the system in a nearly random distribution which, according to information theory [2], maximizes the amount of information that can be cooperatively propagated in a signaling event because it is not biased in any one direction as in a more ordered system. Together the weak cooperativity of lipid molecules and their nearly random distribution suggest that the membrane constitutes a major information transducer in the cell.

To transduce the vast array of information encoded within the mosaic membrane into the cell interior requires both transient organization of the signaling lipid species and a recipient protein that further senses and propagates the message. A lipid mixture near a critical point, teetering on a phase boundary between random distribution and a restricted domain, could fulfill the first requirement. Domain formation can increase the probability of a particular signaling event for instance, by providing platforms for protein–protein interactions that initiate the intracellular portion of a signaling cascade. In this sense, transient order in lipid organization allows for a discrete message to be propagated at levels above background thermal noise, or in other words, above the milieu of all other possible messages encoded in the membrane lipid distribution.

Subsequent detection and propagation of such a wide range of membrane-encoded messages would seem to require a protein with complementary features; the protein would need to have a tendency towards random distributions so as to maximize the amount and diversity of information that can be recognized and propagated. Intrinsically

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disordered proteins are one well-known class of proteins with this tendency. Intrinsically disordered proteins are natively unfolded and have significant structural and conformational plasticity making them uniquely sensitive to differences in the local environment, not unlike the near random but pliable membrane surface. Moreover, intrinsic disorder can facilitate propagation of information within the body of a protein [3]. When we measured the stability of a specific class of proteins by thermal denaturation we found that these proteins, whose functional role is directly linked to membrane biology, have features of intrinsic disorder; these proteins retain some secondary structure, but are marginally stable (hypostable) or nearly disordered [4–6]. Specifically, this seems to be the case with C2 domains of synaptotagmin I (Syt I) in neurons (involved in exocytosis) and the C2 domains of dysferlin (Dys) in muscle (responsible for sensing and repairing membrane damage). Currently there are 14,496 C2 domains annotated in 9,258 protein sequences within the SMART non-redundant database [7]. It is unknown if this marginally stable behavior is a general feature of C2 domains but denaturations carried out previously on various C2 domains from protein kinase C also converge on this finding [8]. Moreover, when we denatured the C2A domain of cotton Syt I, we found it had the same hallmarks as human Syt I, suggesting potential conservation across phylogenetic kingdoms.

A new potential rule for membrane signaling emerges when we simultaneously consider the near disordered nature of membrane lipids and membrane-sensing proteins, namely the maximum flow of signaling information through the membrane and into the cell is optimized by cooperation of the two near-random distributions of membrane lipids and proteins. Indeed, when lipid species diversity and protein intrinsic disorder are compared, both increase with organism complexity [9,10]. While at first glance, this hypothesis may seem to imply unidirectionality of information flow (a marginally stable protein only decodes the information of the membrane), the mechanism applies in both directions. Protein interactions with membranes can induce membrane domain formation (order) if the protein has specificity for some of the lipids and these lipids are distributed non-ideally [11,12]. If, however, the protein's interaction with a membrane depends on intracellular signals (such as calcium ion), then the protein becomes a means to relay intracellular conditions back to the membrane potentially for amplification *via* weak cooperative lipid–lipid interactions. The end result is a reciprocal exchange of information.

Here we explore our hypothesis that the distribution of lipids within the eukaryotic membrane is coupled to interactions with weakly stable but structured proteins to transduce and modulate signaling information. We first use experimental data from the literature on membrane phospholipid compositions in stimulated and unstimulated mast cells to calculate information content encoded within different lipid compositions (excluding cholesterol as a component) as well as their associated acyl chains and head groups [13]. We then complement these findings with recent and new measurements of protein stability and membrane disruption in which membrane lipid composition (and thus information content) is a variable. We find that C2 domain stabilities are highly sensitive to lipid compositions and undergo correlated changes with membrane information content.

## 2. Materials and methods

### 2.1. Protein constructs

The C2 domains studied were constructs derived from human Syt I, cotton Syt I, and Dys. The only C2A construct of human Syt I used for experiments in this study contained residues 96–265. In the top half of Table 3, thermodynamic parameters are reported for a shorter human Syt I C2A construct containing residues 140–265. Additional residues in the 96–265 C2A construct correspond to the region between C2A and Syt I's single transmembrane helix. The human Syt I C2B domain included residues 272–422. The constructs from Dys include two different isoforms of the C2A domain: the canonical C2A domain (C2A wild type/C2A) and the variant resulting from alternative splicing of the first exon (C2Av1). Of the two C2 domains in cotton Syt I, only the C2A domain was studied. Purification of these constructs was carried out as previously described [4–6].

### 2.2. Preparation of lipid samples

All lipids were purchased from Avanti Polar Lipids (Birmingham, AL). Samples without cholesterol were prepared as previously described [6]. Cholesterol-containing samples were prepared by aliquoting lipid stocks into a 4:1 mixture of chloroform:methanol followed by rotary evaporation using a Buchi R-215 at a temperature between 50 and 60 °C. The lipid films were then placed under vacuum for a minimum of 8 h to remove excess solvent and hydrated with the appropriate buffer. LUVs were prepared by hand extrusion using a 0.1 μm filter. SUVs were prepared through multiple rounds of extrusion with filters of gradually smaller pore sizes ending with a 0.03 μm pore size. Lipid species used in the preparation of the LUVs included 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (16:0,18:1PC or POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (16:0,18:1PS or POPS), as well as those shown in Table 1, including the various acyl chain compositions of phoshphatidylethanolamine (PE), phsophatidylinositol (PI), and phsophatidylserine (PS).

### 2.3. Differential scanning calorimetry (DSC)

DSC experiments as well as both scan rate and concentration dependent controls were performed on a NanoDSC (TA Instruments, New Castle, DE) as described previously [4–6]. All scans were conducted in chelexed 20 mM MOPS, and 100 mM KCl, pH 7.5 using saturating ligand concentrations. All scans with lipid contained one of the following: 1) LUVs of 60:40 POPC:POPS; 2) LUVs of (80:20):30 (POPC:POPS):cholesterol in which 30 mol% cholesterol was doped on top of a mixture of POPC:POPS (80:20); 3) LUVs with the mixture shown in Table 1 plus 45 mol% cholesterol doped in; or 4) SUVs composed of mixture 3. The concentration of the calcium stock solution used for all scans was verified using both a calcium ion selective electrode (ThermoScientific) and a BAPTA chelating assay (Invitrogen/Molecular Probes, Eugene, OR). The concentration of all lipid stock solutions was confirmed through a phosphate assay according to standard protocols [6]. For the experiments reported in this study the human Syt I C2A construct was found to have an average reversibility of 93% except in the presence of LUVs composed of the membrane domain forming mixture in which the reversibility was found to be 46%. For a comparison between consecutive denaturation scans, please see Supplementary data. For a justification of the thermodynamic parameters reported for the other domains discussed please see references [4–6].

### 2.4. Tryptophan fluorescence (TF)

TF experiments were performed on a Lifetime Spectrometer (Fluorescence Innovations, Bozeman, MT) using nanomolar protein concentrations as previously described [4,6]. No time-resolved measurements were made; instead the integrated intensity of the lifetime decay was used to monitor intrinsic tryptophan fluorescence (excitation and emission wavelengths of 295 and 340 nm, respectively). Buffers, $Ca^{2+}$ stocks, and lipid samples used were identical to that described above in Section 2.3. Percent reversibility was measured by comparing the integrated fluorescence lifetime intensity of the sample before heating and after cooling. Due to the exceptionally low stability of the Dys and cotton Syt I C2A domains, no change in tryptophan fluorescence could be detected for these constructs. As a result, only the Syt I C2 domains were studied using this method.