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Sphingolipids and membrane targets for therapeutics

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Lipids and membranes are often strongly altered in various diseases and pathologies, but are not often targeted for therapeutic advantage. In particular, the sphingolipids are particularly sensitive to altered physiology and have been implicated as important players in not only several rare hereditary diseases, but also other major pathologies, including cancer. This review discusses some potential targets in the sphingolipid pathway and describes how the initial drug compounds have been evolved to create potentially improved therapeutics. This reveals how lipids and their interactions with proteins can be used for therapeutic advantage. We also discuss the possibility that modification of the physical properties of membranes could also affect intracellular signaling and be of therapeutic interest.

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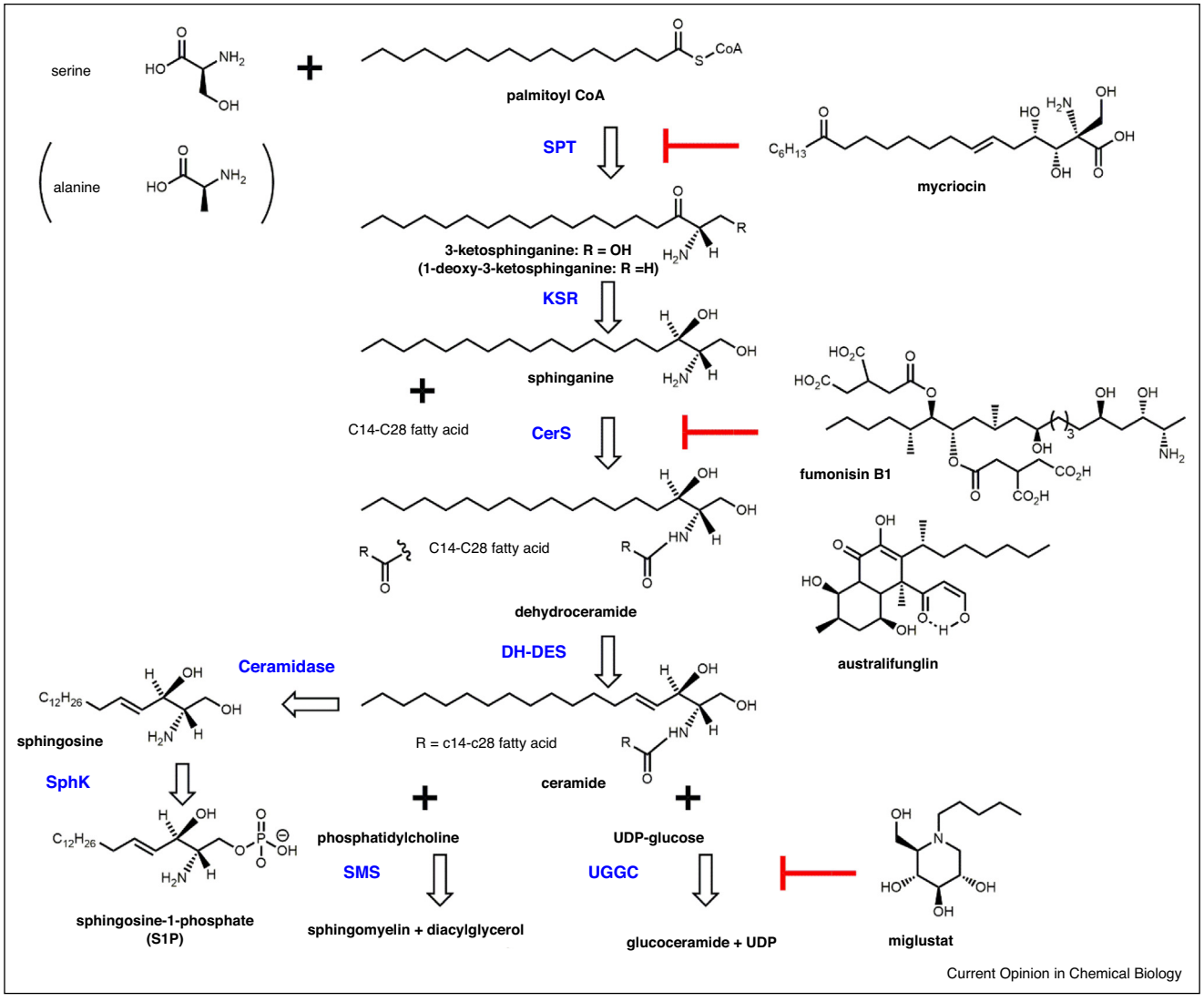
Membrane sphingolipids as targets

Lipids are essential for life, providing a physical membrane barrier between the interior and exterior of cells as well as between intracellular compartments. Lipids are also used as physiological signaling molecules, called bioactive lipids, which fulfil functions of intracellular signaling, as well as intercellular signaling. If the barrier function of membrane lipids were their only function, a simple lipid composition would most likely be sufficient. However, eukaryotic membranes have an enormous lipid diversity, including thousands of individual lipid species [1]. Therefore, membrane lipids are likely to play important roles in regulating protein function, including transmembrane and peripheral membrane proteins, as well as regulating membrane properties required for membrane deformation and vesicular trafficking. Because of their roles in recruiting proteins to membranes and regulating important cellular events like transcription and signal transduction, lipids and the proteins they target could be prime targets for therapeutic intervention. The three major classes of lipids are the glycerophospholipids, sphingolipids, and sterols. Many studies and drugs have targeted the cholesterol synthesis pathway as hypercholesterolemia has been associated with increased risk of cardiovascular disease [2]. Among the glycerophospholipids, only phosphoinositides have been extensively addressed. The inhibition of PI-3 kinases has been developed to treat cancer [3]. Here, we will discuss some aspects of sphingolipid biosynthesis as targets and their roles in membranes, since they are modulated in a large number of diseases and mutations affecting their metabolism have been shown to cause disease. Sphingolipid analogs are therapeutics because of their bioactive properties. However, sphingolipids are also important lipids that regulate membrane properties such as viscosity and tension, which might also make them suitable as novel targets for therapeutic intervention. Sphingolipid degradation also reveals therapeutic targets [4], but this will not be addressed here. Finally, we will explore the burgeoning idea that membranes per se may serve as clinically relevant drug targets.

The sphingolipid biosynthesis pathway begins with the formation of 3-ketosphinganine from serine and palmitoyl-CoA (Figure 1) by serine palmitoyltransferase (SPT). SPT is composed of two major subunits and several small subunits that are most likely involved in its regulation and specificity. The enzyme is also regulated through the action of ORM proteins, which inhibit SPT activity. Myriocin, a natural product which was originally identified with anti-fungal activity [5], has been shown to inhibit sphingolipid biosynthesis [6], affecting intracellular transport of glycosylphosphatidylinositol anchored proteins, before SPT was identified as the target [7]. As sphingolipids, especially ceramides and glucosylceramides accumulate in a wide variety of diseases, sphingolipid biosynthesis is widely viewed as a therapeutic target for many different indications [8] as a large number of patents can be found in a simple search. However, long-term myriocin treatment does not seem to be a feasible therapeutic because of toxic side effects and because its action on SPT is at least partially irreversible [9]. Nevertheless, myriocin may be used to reduce

Figure 1



Sphingolipid biosynthesis pathway. Only steps up until the formation of the first complex sphingolipids are shown. The sites of action of some inhibitors of the pathway are shown.

ischemia-reperfusion injury [10] as a short-term application would be possible. It should be possible to find novel inhibitors of SPT that have less toxicity, especially if reversible, and these could be used for a large number of indications where ceramides and sphingolipids are accumulated leading to unwanted side effects. Inhibition of SPT can be looked at as similar to inhibition of cholesterol production, where statins lower the amounts of an essential lipid by interfering at a key step early in the pathway. Both SPT [11] and HMG-CoA reductase [12] are important regulatory steps in cells. Another similarity between SPT inhibition and HMG-CoA reductase inhibition is that downstream products, although essential, can also be obtained through the diet [13], which can temper the effect of inhibition of synthesis.

Despite a strong preference for serine, SPT can also accept alanine or glycine as substrates, which lead to the production of 1-deoxysphingolipids [14]. Mutations in SPT that decrease the preference for serine and increase the production of 1-deoxysphingolipids are at the origin of the rare disease, Hereditary Sensory and Autonomic Neuropapthy type 1 [15•]. The exact mechanism of neurotoxicity of 1-deoxysphingolipids is still unknown, but understanding of the enzymology underlying the disease has led to a strategy to improve the quality of life in HSAN type I patients [16] by increasing serine in the diet.

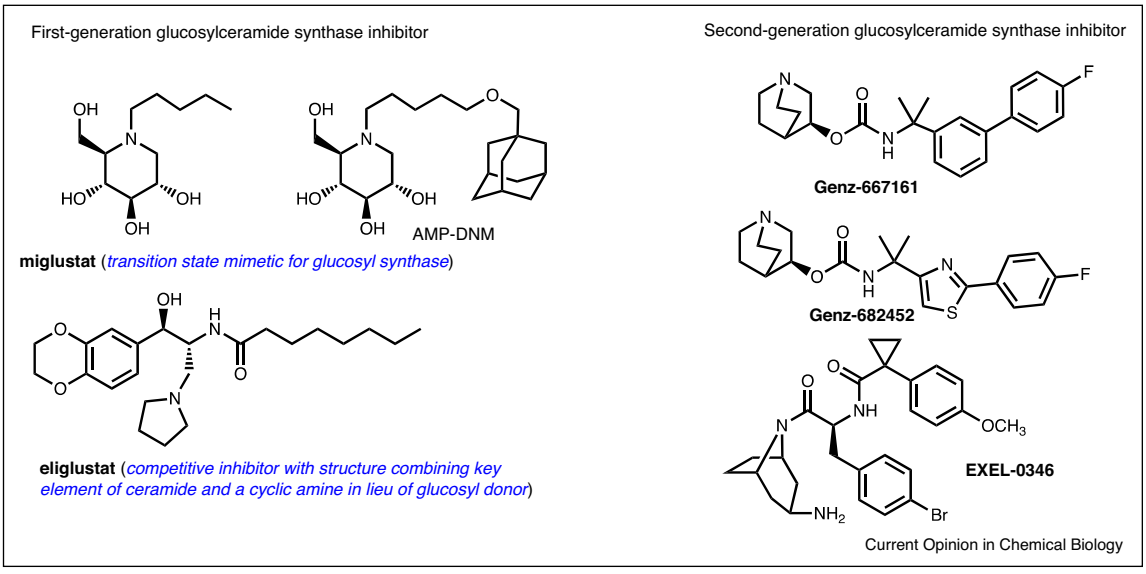
After SPT action, 3-ketosphinganine is converted to sphinganine by 3-ketosphinganine reductase (KSR),

which together with acyl CoA is used by multiple ceramide synthases (CerS) to form dihydroceramide. Mammals have 6 CerS which differ in their substrate specificity, best described for their acyl chain length preferences [17]. Fairly general inhibitors of ceramide synthases, fumonisin B1 [18] and australifungin [19], have been identified, but inhibitors for specific ceramide synthases could be of therapeutic interest. Dihydroceramide is then desaturated by dihydroceramide desaturase (DH-DES) to form ceramide. Many biological properties of ceramides, including the ability to induce apoptosis are dependent upon this desaturation [20]. Therefore, DH-DES has been targeted by small molecule inhibitors [21]. Both dihydroceramides and ceramides can be transported from their site of synthesis in the endoplasmic reticulum to the Golgi compartment [1] where they are converted to sphingomyelin by sphingomyelin synthase (SMS) or glucosylceramide by glucosylceramide synthase (UCGC). In most cell types sphingomyelin is more abundant than glucosylceramide, but the latter is used to form a large series of complex glycosphingolipids with various roles in development, cell–cell interactions and as targets for virus and toxin entry. Inhibition of glucosylceramide synthesis by miglustat is used for the treatment of the rare diseases where cholesterol and glycosphingolipids accumulate in endosomes and lysosomes (see below). Inhibition of SPT or UCGC were also shown in a mouse model to prevent appearance of hepatocellular carcinoma nodules in a mTORC2-driven cancer model [22•]. In the latter model, SPT was inhibited using myriocin treatment, whereas the UCGC was inhibited using shRNA encoded on a viral vector. This study indicates a therapeutic potential for SPT and UCGC inhibitors for fatty hepatocellular carcinoma and perhaps other lipid-driven cancers.

Miglustat (Zavesca, *N*-butyl-deoxynojirimycin: NB-DNJ, Figure 2), an alkyl iminosugar which mimics the transition state of the cationic intermediate in the glycosylation reaction, prevents the accumulation of glucosylceramide. Miglustat is a competitive inhibitor of glucosylceramide synthase. It has been approved for the treatment of Type I Gaucher's disease and Niemann–Pick disease. Interestingly, iminosugars can also bind glucocerebrosidase and act as pharmacological chaperones. Specifically, an analog of miglustat (*N*-nonyl-deoxynojirimycin, NN-DNJ) was shown to rescue lysosomal β-glucosidase activity of N370S, a mutant that is prevalent in Gaucher disease, by stabilizing the protein and enhancing its trafficking to the lysosome [23]. As such, it is a prominent example of a corrector for metabolic disease. An alternative strategy also leveraged on substrate analogs, included functionalities mimicking ceramide [24], led to the discovery of eliglustat (Genz-112638) [25].

Further drug development on this target led researchers at Genzyme to the discovery of heterocyclic pharmacophore deprived of conformationally flexible alkyl chains (Genz-667161 and Genz-682452). Genetic evidence of a correlation between Gaucher disease and the synucleinopathies Parkinson disease [26] as well as Fabry disease (a glycosphingolipid storage disorder caused by the deficient activity of alpha-galactosidase A with cerebrovascular complication) generated an impetus to discover novel brain permeant glucosylceramide synthase inhibitors (miglustat has low brain penetration) [27]. Genz-667161 was recently shown to reduce levels of glucosylceramide and

Figure 2



Structure of representative examples of glucoceramide synthase inhibitors.

glucosylsphingosine in the central nervous system (CNS) of a mouse model, demonstrating target engagement. Furthermore, treatment with Genz-667161 slowed the accumulation of hippocampal aggregates of alpha-synuclein, ubiquitin, and tau, and improved the associated memory deficits [26].

In parallel, an optimized iminosugar (AMP-DNM, Figure 2), a more potent glucosylceramide synthase inhibitor than structurally related miglustat, has been shown to counteract TNF-alpha-induced abnormalities in glycosphingolipid concentrations and reverse abnormalities in insulin signal transduction [28]. A high throughput screening campaign at Exelixis led to the discovery of a different pharmacophore which, following hit optimization, resulted in a low nanomolar inhibitor of glucosylceramide synthase (EXEL-0346) [29].

Signaling sphingolipids

The role of lipids as signaling molecules has long been recognized and their role as messengers in cellular processes, including cell proliferation, apoptosis, metabolism, and migration is well established. Key lipid-modifying enzymes respond to extracellular signals such as growth factors, cytokines or nutrients by changing the composition of these signaling lipids in a complex network harboring multiple nodes of interactions and cross-regulatory mechanisms. Imbalances in this network contribute to inflammation, cancer and metabolic disorders amongst other diseases [30]. Despite the appreciation for their important role, the nondrug-like properties of signaling lipids has deterred and hampered pharmacological approaches to correct imbalances or agonize/antagonize key signaling lipids, favoring drug development on target classes perceived as more druggable. The pursuit of small molecules constrained by some aspects of the 'rules of five' [31] made lipids somewhat outliers in the world of drug discovery.

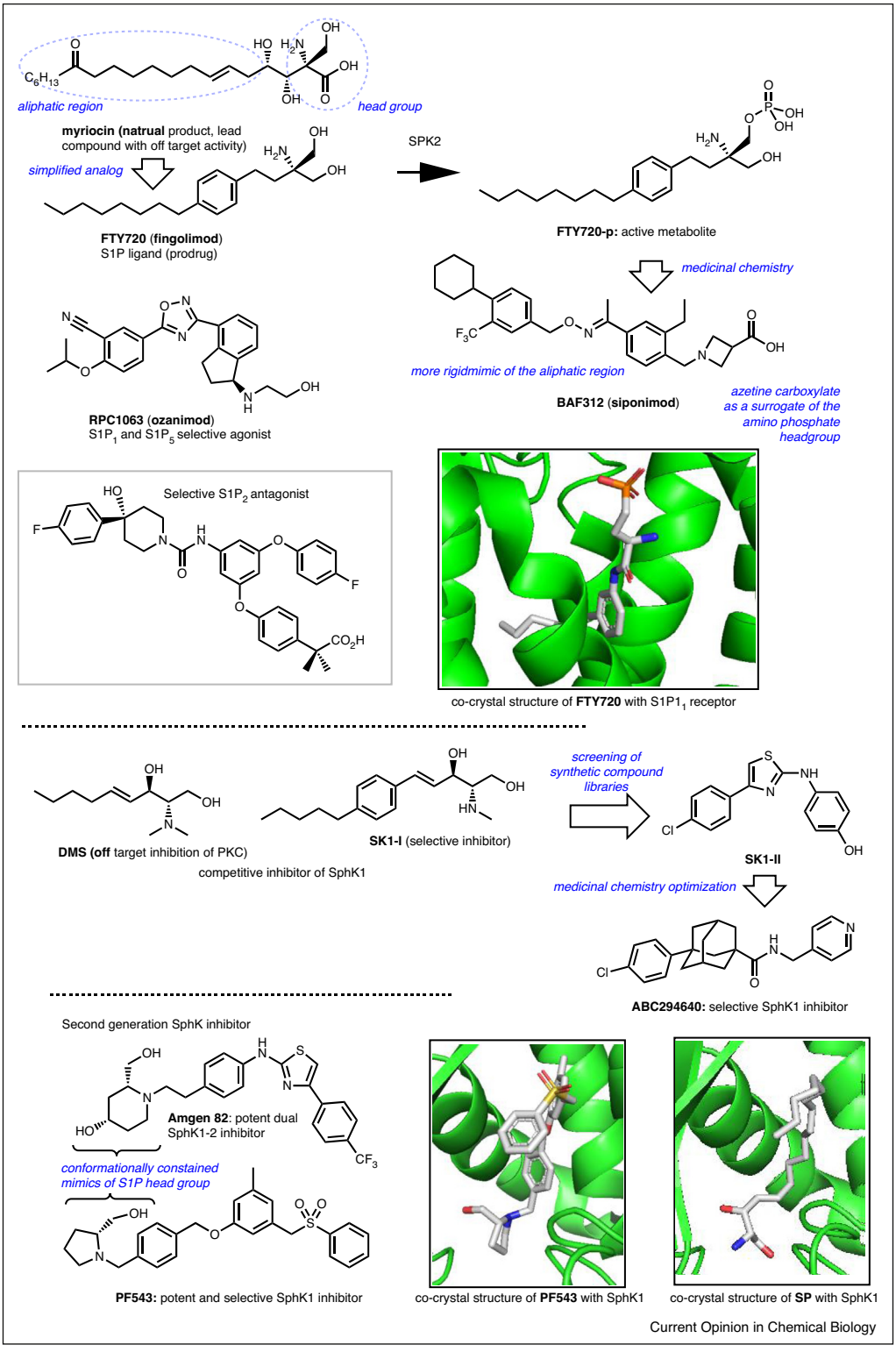
Nonetheless, there have been notable achievements in the field which should encourage more medicinal chemists to venture in the less chartered area of lipid signaling. A common thread through these efforts is that the first-generation inhibitors are generally based on a substrate mimetic with relatively poor potency and selectivity. However, these inhibitors play a crucial role in establishing the drugability of the target. Accrued efforts on a given target yields more drug-like small molecules, which are chemically more distinct from the substrate and provide better efficacy.

The fortuitous discovery of FTY720 (fingolimod, Figure 3) as an immunosuppressant and the latter discovery that its phosphorylated metabolite (FTY720-P) binds to S1P receptor, thus promoting receptor internalization and degradation, instigated tremendous research in the area [32]. The immunosuppressive effect stems from the depletion of S1P receptor on lymphocytes resulting in their sequestration in lymphoid organs [19]. The clinical success of FTY720 for the treatment of multiple sclerosis and its applicability to other indications requiring immunomodulation prompted significant medicinal chemistry efforts to develop next generation inhibitors. Notably, efforts to design compounds that would not require metabolic processing (phosphorylation by sphingosine kinase) led, for example, to the development of an azetidine carboxylate as a surrogate for the amino phosphate head group of FTY720-P. This azetidine carboxylate was used in many inhibitors including the clinical candidate BAF312 (siponimod) which was recently shown to be effective in a phase III trial in patients with secondary progressive multiple sclerosis (SPMS) [33]. Another characteristic of second-generation inhibitors was the replacement of the unstructured alkyl chain of FTY720 with aryl or heteroaromatic groups. Compound development was also aided by the co-crystal structure of FTY720-P with S1P receptor [34••]. The structure provided a detailed view of the molecular recognition and hydrophobic volume required for activation of the S1P receptor. It is interesting to note that access to the binding pocket from the extracellular environment is blocked by the amino terminus and extracellular loops of the receptor. Access is gained by the ligand diffusing in the phospholipid bilayer and entering laterally between helices within the transmembrane region of the receptor [34••]. Further medicinal chemistry optimization demonstrated that it was possible to obtain a direct agonist of S1P without a polar acid head group such as in ozanimod which only contains a polar amino alcohol head group. Notably, this compound displayed excellent selectivity against S1P receptor type1 and S1P receptor type 5 versus S1P receptor type 3 [35]. The type 3 receptor is associated with toxicity.

While the majority of compounds designed against S1P receptor type 1 also target other subtypes of S1P receptors, none show strong binding to S1P receptor type 2. Genetic evidence suggests that selectively targeting this receptor subtype may prove beneficial for the treatment of hypertension and targeted efforts toward S1P receptor type 2 antagonists yielded the discovery of an orally available antagonist [36].

As an alternative approach to targeting S1P receptors, inhibition of sphingosine kinase has also been pursued. S1P is produced by sphingosine kinase (two subtypes in mammals: SphK1 and SphK2). Furthermore, in addition to the well characterized interaction of S1P with cell surface S1P receptors, there is mounting evidence that S1P also has intracellular targets [37]. SphK is a key regulator of S1P levels and the S1P:Sph/ceramide ratio. Also, ceramide synthase2 (CerS2) is inhibited by S1P [38]. S1P has also been reported to regulate the histone acetylation in the nucleus [39]. Increases in S1P levels have been linked to diseases including sickle cell disease, cancer, and fibrosis. The development of SphK inhibitors parallels those of S1P receptor agonists with the first generation inhibitors (such as *N*,*N*-dimethylsphingosine: DMS [40] and SK1-I [41]) closely mimicking the

Figure 3



Representative examples of S1P modulators (top) and SphK inhibitors (bottom).

structure of sphingosine and acting as competitive inhibitors. Screening of synthetic compound libraries led to the discovery of SK1-II with a drug-like heterocyclic core [42]. Further optimization of the compound yielded ABC294640 [43], a first-in-class orally available inhibitor of SK2, which is currently in clinical trials (Phase I, patients with solid tumors) [44]. The publication of co-crystal structure of SphK1 with S1P [45] and first generation inhibitor SK1-II enabled structure-based design of potent second generation inhibitors such as Amgen 82 (dual SphK1-2 inhibitor) wherein the hydroxylated piperidine head group mimics the head group of S1P. Researchers at Pfizer identified the most potent SphK1 inhibitor (PF-543) by combining fragments from two different screening hits [46•]. The co-crystal structure of this inhibitor with SphK1 clearly show a good overlap between the positioning of the aryl groups of PF-543 and the alkyl chain of S1P as well as the respective head groups [47].

Membranes as drug targets

Thus far we have highlighted the roles of sphingolipids species in signaling and disease. Using small molecules to target the biosynthetic enzymes that generate these lipids or the protein interfaces that interact with these lipids is an obvious, but under developed, avenue of future drug design. Indeed, targeting proteins is presently common practice and of the 1578 FDA-approved drugs, 96% target proteins [37]. However, there is no *a priori* reason to limit drug-target-space to proteins. Lipids, like amino acids, also assemble into macromolecular structures such as lipid droplets and membranes. In this final section we ask: might these lipid-based macromolecular structures also be directly targetable for therapeutic gain?

As elaborated above, cells dynamically adjust both the chemical composition as well as biophysical aspects of their membranes. Membrane lipid composition varies between organisms, cell types, organelles, leaflets and even within a leaflet by partitioning into subdomains and how these specific chemical compositions affect the function of membrane proteins is only now starting to be addressed. This is an important avenue of research as loss of membrane lipid composition homeostasis is correlated with various diseases [1]. Furthermore, the discrete biophysical properties of different membranes could, in principle, present privileged nodes for therapeutic intervention.

Membrane tension is defined as the in-plane counteracting force to surface expansion. In some experimental settings tension can be measured, often by the tedious process of determining the force required to extract a membrane tube from a membrane surface. *In vitro*, membrane tension is highly variable ranging from 10^{-8} N/m to 10^{-2} N/m [48,49], whereas *in vivo*, when measurable, plasma membrane tension is held to much narrowing confines, between 10^{-5} N/m to 5×10^{-4} N/m, with cell rupture occurring at tensions $>10^{-2}$ N/m [50–53]. Consistently, all cell types analyzed were found to actively counteract perturbations in plasma membrane tension demonstrating that this is indeed a tightly controlled biophysical parameter [54–56]. Tension *per se* can serve to integrate and transfer information within cells and tissues [57–59], although recent studies have unexpectedly suggested that membrane tension may, in fact, only be transmitted over very short distances *in vivo* [60,61]. How plasma membrane tension is sensed and regulated remains poorly understood, due, in large part, to a lack of appropriate tools and to the inherent challenges incurred by the interdisciplinary approaches needed to address these questions. More egregious is our lack of understanding of tension in endo-membranes, which are, of course, even less experimentally accessible.

As shown by caveolinopathies, loss of membrane tension homeostasis seems to lead to disease. Caveolae are small pits found in the plasma membrane of many mammalian cells. Their core structural protein components are the caveolins and cavins while the Eps15 homology domain (EHD) proteins and pacsin/Syndapin proteins are additionally required for efficient caveolae formation/stability. Adipocytes, endothelial cells and muscle cells show a particularly high density of caveolae which may sequester up to 50% of total membrane surface area. Consistent with this observation, caveolae are now believed to play a major role in mechanoprotection, although other tissue specific functions have also been proposed [62•]. Direct measurements demonstrated that caveolae-flattening helps buffer plasma membrane tension increases in cells experiencing hypo-osmotic shock [53]. Furthermore, relative to controls, cells and tissues lacking caveolae present extensive membrane damage upon exposure to physical stress. Patients harboring mutations that affect caveolae formation present various diseases (caveolinopathies) including muscular dystrophies, cardiomyopathies, pulmonary arterial hypertension and glaucoma — diseases linked by the fact that cells in affected tissues are highly susceptibility to plasma membrane rupture [62•,63]. Collectively, these observations demonstrate a potentially causal link between loss of plasma membrane tension regulation and human disease and thus provoke the question: can membrane tension be targeted for therapeutic gain?

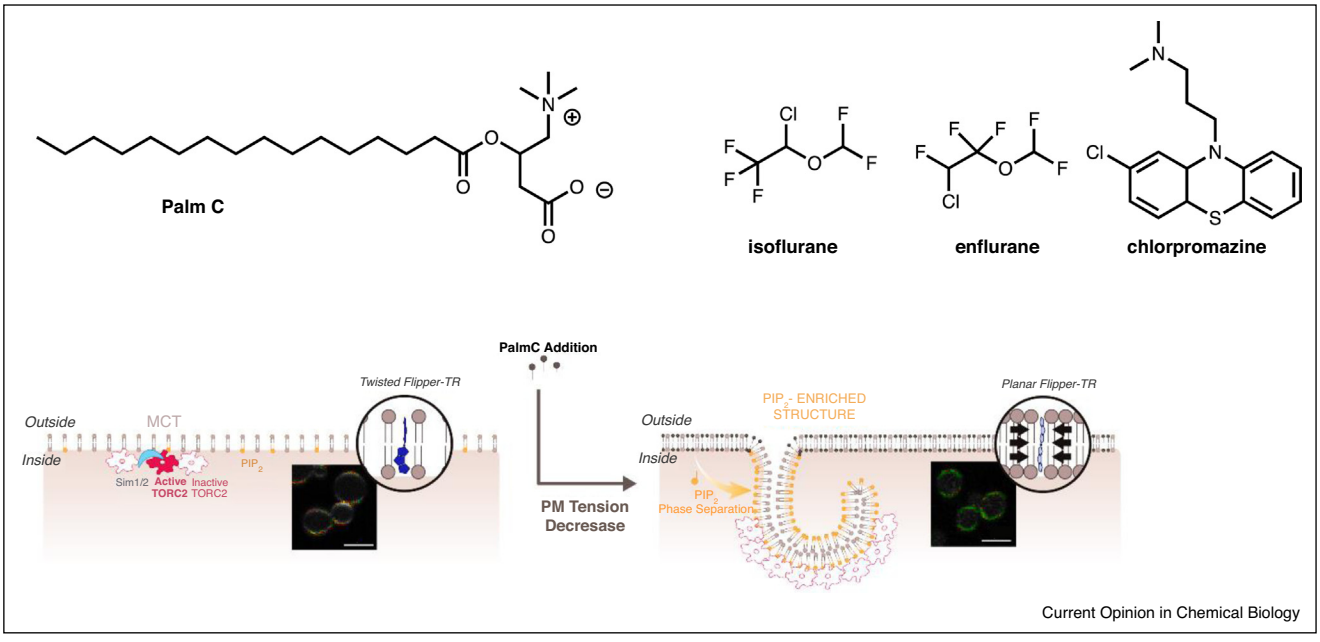
The Target Of Rapamycin (TOR) is an atypical eukaryote protein kinase that assembles into two, distinct multiprotein complexes known as TORC1 and TORC2 [64]. In 2012, it was reported that blocking sphingolipid biosynthesis in yeast strongly activates TORC2 but not TORC1 [65•]. Furthermore, hypo-osmotic shock and physical 'pulling' of the plasma membrane of yeast spheroplasts also hyperactivated TORC2. Although it could not be measured at that time, all three of these otherwise

orthogonal manipulations presumably caused an increase in the tension of the plasma membrane (by respectively reducing the amount of lipid that can contribute to the membrane, increasing turgor pressure, and, physically stretching the membrane). TORC2 activation by these manipulations requires the translocation of the paralogous BAR-domain proteins Slm1 and Slm2 from caveolae-like membrane invaginations known as eisosomes into plasma membrane domains containing TORC2 [65•]. Conversely, the loss of membrane tension triggered by hyper-osmotic shock leads to TORC2 inactivation.

As a means to characterize the regulatory events upstream of TORC2, a high throughput screen to identify small molecules that antagonize TORC2 signaling was performed. This screen led to the identification of palmitoyl carnitine (PalmC, Figure 4 top) [66••]. Given its detergent-like structure and the fact that TORC2 is regulated downstream of plasma membrane tension, it was tested whether the primary target of PalmC was the yeast plasma membrane. Indeed, this appears to be the case: addition of PalmC to giant unilamellar vesicles *in vitro* provokes an immediate increase in membrane surface area, and, similarly to hyper-osmotic shock, addition of PalmC to yeast cells triggers an immediate decrease in plasma membrane tension as readout by the fluorescent membrane tension probe Flipper-TR (fluorescent lipid tension reporter) [67••] and inhibition of TORC2 signaling (Figure 4 bottom).

These results with PalmC, albeit in the model eukaryote *Saccharomyces cerevisiae*, lend validation to the concept that the biophysical properties of membranes serve as eligible drug targets (in this case as a means to inhibit TORC2 signaling). Attempting to identify drugs that target membranes might sound rather unorthodox; however, we note that several clinically used drugs are thought to function by altering membrane 'fluidity'. These include the volatile anesthetics isoflurane, halothane, enflurane, sevoflurane, and methoxyflurane [68], and the antipsychotic drug chlorpromazine [69] (Figure 4 top). We note, however, that the precise mode of action of these compounds remains controversial [70,71]. While there is a broad array of biophysical methods to study the effect of small molecules on proteins (affinity, promotion or inhibition of protein–protein interactions), it is not the case for the impact of small molecules on membranes. The development of probes, such as Flipper-TR, that can specifically measure changes in the biophysical properties of membranes *in situ* will certainly help in this direction. Including membranes (plasma membranes as well as endomembranes) as viable targets would open new opportunities in drug-discovery and potentially provide an inroad to address hitherto unmet medical needs. It is clear that some drugs partition in the membrane and, as discussed for FTY720, gain access to their target protein through lateral diffusion within a membrane. Membrane composition and properties may affect the partitioning and diffusion. While these questions are difficult to

Figure 4



Membranes can be targeted by small molecules. (Top) Structure of palmitoylcarnitine (PalmC) and other small molecules that potentially interact with membranes. (Bottom) Cartoon illustrating that PalmC intercalates into the plasma membrane of yeast cells, triggering a massive drop in membrane tension as visualized by changes in Flipper-TR fluorescence lifetime imaging (insets). Drop in membrane tension leads to phase separation of Phosphatidylinositol 4,5-bisphosphate (PIP2) and TORC2 inhibition. See Ref. [66••] for details.

address with current technologies, this challenge is an opportunity to innovate.

Conclusions

It is abundantly clear that dysregulation of lipid biosynthesis and the loss of membrane homeostasis lead to dire medical consequences. To better understand the molecular roots behind these lipid/membrane-related diseases new tools to study the unique composition and biophysical properties of specific membranes are being developed, such as Secondary ion mass spectrometry (nanoSIMS) [72] and the Flipper-TR probe discussed above [67••], but more are needed. Imaging technologies, like the fluorsescent membrane tension probe [67••], that can report on membrane changes would empower our ability to link such changes to disease states. Ultimately, these tools will help guide the design, characterization, and, ideally clinical validation of novel therapeutics that correct lipid imbalances and/or loss of homeostatic control of the biophysical set points of various membranes. The progression into the clinic and the approval of several small molecules targeting sphingolipid processing enzymes and receptors clearly highlight the advances that have been made. To date, there is still a lack of small molecules that target lipid transport proteins.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Harayama T, Riezman H: **Understanding the diversity of membrane lipid composition**. *Nat Rev Mol Cell Biol* 2018, **19**:281-296.

2. Nair P: **Brown and Goldstein: the cholesterol chronicles**. *Proc Natl Acad Sci U S A* 2013, **110**:14829-14832.

3. Pons-Tostivint E, Thibault B, Guillermet-Guibert J: **Targeting PI3K signaling in combination cancer therapy**. *Trends Cancer* 2017, **3**:454-469.

4. Canals D, Perry DM, Jenkins RW, Hannun YA: **Drug targeting of sphingolipid metabolism: sphingomyelinases and ceramidases**. *Br J Pharmacol* 2011, **163**:694-712.

5. Kluepfel D, Bagli J, Baker H, Charest MP, Kudelski A: **Myriocin, a new antifungal antibiotic from Myriococcum albomyces**. *J Antibiot (Tokyo)* 1972, **25**:109-115.

6. Horvath A, Sutterlin C, Manningkrieg U, Movva NR, Riezman H: **Ceramide synthesis enhances transport of Gpi-anchored proteins to the Golgi-apparatus in yeast**. *EMBO J* 1994, **13**:3687-3695.

7. Miyake Y, Kozutsumi Y, Nakamura S, Fujita T, Kawasaki T: **Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, Isp-1/myriocin**. *Biochem Biophys Res Commun* 1995, **211**:396-403.

8. Fox TE, Finnegan CM, Blumenthal R, Kester M: **The clinical potential of sphingolipid-based therapeutics**. *Cell Mol Life Sci* 2006, **63**:1017-1023.

9. Wadsworth JM, Clarke DJ, McMahon SA, Lowther JP, Beattie AE, Langridge-Smith PR, Broughton HB, Dunn TM, Naismith JH, Campopiano DJ: **The chemical basis of serine palmitoyltransferase inhibition by myriocin**. *J Am Chem Soc* 2013, **135**:14276-14285.

10. Reforgiato MR, Milano G, Fabrias G, Casas J, Gasco P, Paroni R, Samaja M, Ghidoni R, Caretti A, Signorelli P: **Inhibition of ceramide de novo synthesis as a postischemic strategy to reduce myocardial reperfusion injury**. *Basic Res Cardiol* 2016, **111**:12.

11. Breslow DK, Weissman JS: **Membranes in balance: mechanisms of sphingolipid homeostasis**. *Mol Cell* 2010, **40**:267-279.

12. Brown MS, Goldstein JL: **Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth**. *J Lipid Res* 1980, **21**:505-517.

13. Vesper H, Schmelz EM, Nikolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill AH Jr: **Sphingolipids in food and the emerging importance of sphingolipids to nutrition**. *J Nutr* 1999, **129**:1239-1250.

14. Zitomer NC, Mitchell T, Voss KA, Bondy GS, Pruett ST, Garnier-Amblard EC, Liebeskind LS, Park H, Wang E, Sullards MC *et al.*: **Ceramide synthase inhibition by fumonisin B1 causes accumulation of 1-deoxysphinganine: a novel category of bioactive 1-deoxysphingoid bases and 1-deoxydihydroceramides biosynthesized by mammalian cell lines and animals**. *J Biol Chem* 2009, **284**:4786-4795.

15. • Penno A, Reilly MM, Houlden H, Laura M, Rentsch K, Niederkofler V, Stoeckli ET, Nicholson G, Eichler F, Brown RH Jr *et al.*: **Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids**. *J Biol Chem* 2010, **285**:11178-11187.
This article identifies two neurotoxic sphingolipids, 1-deoxysphingolipids, that accumulate in the rare disease HSAN type I due to modification of the enzymatic specificity of serine palmitoyltransferase.

16. Garofalo K, Penno A, Schmidt BP, Lee HJ, Frosch MP, von Eckardstein A, Brown RH, Hornemann T, Eichler FS: **Oral L-serine supplementation reduces production of neurotoxic deoxysphingolipids in mice and humans with hereditary sensory autonomic neuropathy type 1**. *J Clin Invest* 2011, **121**:4735-4745.

17. Levy M, Futerman AH: **Mammalian ceramide synthases**. *IUBMB Life* 2010, **62**:347-356.

18. Merrill AH Jr, Sullards MC, Wang E, Voss KA, Riley RT: **Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins**. *Environ Health Perspect* 2001, **109**:283-289.

19. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C *et al.*: **Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists**. *Science* 2002, **296**:346-349.

20. Hannun YA, Obeid LM: **Sphingolipids and their metabolism in physiology and disease**. *Nat Rev Mol Cell Biol* 2018, **19**:175-191.

21. Casasampere M, Ordonez YF, Pou A, Casas J: **Inhibitors of dihydroceramide desaturase 1: therapeutic agents and pharmacological tools to decipher the role of dihydroceramides in cell biology**. *Chem Phys Lipids* 2016, **197**:33-44.

22. • Guri Y, Colombi M, Dazert E, Hindupur SK, Roszik J, Moes S, Jenoe P, Heim MH, Riezman I, Riezman H *et al.*: **mTORC2 promotes tumorigenesis via lipid synthesis**. *Cancer Cell* 2017, **32**:807-823 e812.
A mouse hepatocellular carcinoma model system showed that mTORC2-dependent increases in lipid synthesis proceeds and is required for development of tumors.

23. Sawkar AR, Cheng WC, Beutler E, Wong CH, Balch WE, Kelly JW: **Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease**. *Proc Natl Acad Sci U S A* 2002, **99**:15428-15433.

24. Lee L, Abe A, Shayman JA: **Improved inhibitors of glucosylceramide synthase**. *J Biol Chem* 1999, **274**:14662-14669.

25. McEachern KA, Fung J, Komarnitsky S, Siegel CS, Chuang WL, Hutto E, Shayman JA, Grabowski GA, Aerts JMFG, Cheng SH *et al.*: **A specific and potent inhibitor of glucosylceramide synthase for substrate inhibition therapy of Gaucher disease**. *Mol Genet Metab* 2007, **91**:259-267.

26. Sardi SP, Clarke J, Kinnecom C, Tamsett TJ, Li LY, Stanek LM, Passini MA, Grabowski GA, Schlossmacher MG, Sidman RL *et al.*: **CNS expression of glucocerebrosidase corrects alpha-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy**. *Proc Natl Acad Sci U S A* 2011, **108**:12101-12106.

27. Ashe KM, Budman E, Bangari DS, Siegel CS, Nietupski JB, Wang B, Desnick RJ, Scheule RK, Leonard JP, Cheng SH *et al.*: **Efficacy of enzyme and substrate reduction therapy with a novel antagonist of glucosylceramide synthase for Fabry disease**. *Mol Med* 2015, **21**:389-399.

28. Aerts JM, Ottenhoff R, Powlson AS, Grefhorst A, van Eijk M, Dubbelhuis PF, Aten J, Kuipers F, Serlie MJ, Wennekes T *et al.*: **Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity**. *Diabetes* 2007, **56**:1341-1349.

29. Richards S, Larson CJ, Koltun ES, Hanel A, Chan V, Nachtigall J, Harrison A, Aay N, Du HW, Arcalas A *et al.*: **Discovery and characterization of an inhibitor of glucosylceramide synthase**. *J Med Chem* 2012, **55**:4322-4335.

30. Wymann MP, Schneiter R: **Lipid signalling in disease**. *Nat Rev Mol Cell Biol* 2008, **9**:162-176.

31. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: **Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings**. *Adv Drug Deliv Rev* 1997, **23**:3-25.

32. Dyckman AJ: **Modulators of sphingosine-1-phosphate pathway biology: recent advances of sphingosine-1-phosphate receptor 1 (S1P(1)) agonists and future perspectives**. *J Med Chem* 2017, **60**:5267-5289.

33. Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, Vermersch P, Arnold DL, Arnould S, Scherz T *et al.*: **Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study**. *Lancet* 2018, **391**:1263-1273.

34. •• Hanson MA, Roth CB, Jo EJ, Griffith MT, Scott FL, Reinhart G, Desale H, Clemons B, Cahalan SM, Schuerer SC *et al.*: **Crystal structure of a lipid G protein-coupled receptor**. *Science* 2012, **335**:851-855.

This structure provides a molecular rational for receptor subtype selectivity by different agonist and is a landmark for a rational drug design.

35. Scott FL, Clemons B, Brooks J, Brahmachary E, Powell R, Dedman H, Desale HG, Timony GA, Martinborough E, Rosen H *et al.*: **Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P(1)) and receptor-5 (S1P(5)) agonist with autoimmune disease-modifying activity**. *Br J Pharmacol* 2016, **173**:1778-1792.

36. Kusumi K, Shinozaki K, Yamaura Y, Hashimoto A, Kurata H, Naganawa A, Ueda H, Otsuki K, Matsushita T, Sekiguchi T *et al.*: **Discovery of novel S1P(2) antagonists. Part 2: Improving the profile of a series of 1,3-bis(aryloxy)benzene derivatives**. *Bioorg Med Chem Lett* 2015, **25**:4387-4392.

37. Santos WL, Lynch KR: **Drugging sphingosine kinases**. *ACS Chem Biol* 2015, **10**:225-233.

38. Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH, Futerman AH: **Characterization of ceramide synthase 2: tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate**. *J Biol Chem* 2008, **283**:5677-5684.

39. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S *et al.*: **Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate**. *Science* 2009, **325**:1254-1257.

40. Yatomi Y, Ruan FQ, Megidish T, Toyokuni T, Hakomori SI, Igarashi Y: ***N,N*-dimethylsphingosine inhibition of sphingosine kinase and sphingosine 1-phosphate activity in human platelets**. *Biochemistry* 1996, **35**:626-633.

41. Paugh SW, Paugh BS, Rahmani M, Kapitonov D, Almenara JA, Kordula T, Milstien S, Adams JK, Zipkin RE, Grant S *et al.*: **A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia**. *Blood* 2008, **112**:1382-1391.

42. French KJ, Schrecengost RS, Lee BD, Zhuang Y, Smith SN, Eberly JL, Yun JK, Smith CD: **Discovery and evaluation of inhibitors of human sphingosine kinase**. *Cancer Res* 2003, **63**:5962-5969.

43. French KJ, Zhuang Y, Maines LW, Gao P, Wang WX, Beljanski V, Upson JJ, Green CL, Keller SN, Smith CD: **Pharmacology and antitumor activity of ABC294640, a selective inhibitor of sphingosine kinase-2**. *J Pharmacol Exp Ther* 2010, **333**:129-139.

44. Britten CD, Garrett-Mayer E, Chin SH, Shirai K, Ogretmen B, Bentz TA, Brisendine A, Anderton K, Cusack SL, Maines LW *et al.*: **A phase I study of ABC294640, a first-in-class sphingosine kinase-2 inhibitor, in patients with advanced solid tumors**. *Clin Cancer Res* 2017, **23**:4642-4650.

45. Wang Z, Min X, Xiao SH, Johnstone S, Romanow W, Meininger D, Xu HD, Liu JS, Dai J, An SZ *et al.*: **Molecular basis of sphingosine kinase 1 substrate recognition and catalysis**. *Structure* 2013, **21**:798-809.

46. • Schnute ME, McReynolds MD, Kasten T, Yates M, Jerome G, Rains JW, Hall T, Chrencik J, Kraust M, Cronin CN *et al.*: **Modulation of cellular S1P levels with a novel, potent and specific inhibitor of sphingosine kinase-1**. *Biochem J* 2012, **444**:79-88.

This article reports the first potent and selective inhibitor of SphK1 (100-fold selectivity over SphK2). This inhibitor highlighted that specific inhibition of SphK1 had no effect on the cellular proliferation and survival, despite a dramatic change in the cellular S1P/sphingosine ratio.

47. Wang J, Knapp S, Pyne NJ, Pyne S, Elkins JM: **Crystal structure of sphingosine kinase 1 with PF-543**. *ACS Med Chem Lett* 2014, **5**:1329-1333.

48. Pecreaux J, Dobereiner HG, Prost J, Joanny JF, Bassereau P: **Refined contour analysis of giant unilamellar vesicles**. *Eur Phys J E Soft Matter* 2004, **13**:277-290.

49. Morlot S, Galli V, Klein M, Chiaruttini N, Manzi J, Humbert F, Dinis L, Lenz M, Cappello G, Roux A: **Membrane shape at the edge of the dynamin helix sets location and duration of the fission reaction**. *Cell* 2012, **151**:619-629.

50. Dai J, Sheetz MP, Wan X, Morris CE: **Membrane tension in swelling and shrinking molluscan neurons**. *J Neurosci* 1998, **18**:6681-6692.

51. Evans E, Heinrich V, Ludwig F, Rawicz W: **Dynamic tension spectroscopy and strength of biomembranes**. *Biophys J* 2003, **85**:2342-2350.

52. Raucher D, Sheetz MP: **Characteristics of a membrane reservoir buffering membrane tension**. *Biophys J* 1999, **77**:1992-2002.

53. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, Abankwa D, Stan RV, Butler-Browne G, Vedie B, Johannes L *et al.*: **Cells respond to mechanical stress by rapid disassembly of caveolae**. *Cell* 2011, **144**:402-413.

54. Dai J, Sheetz MP: **Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers**. *Biophys J* 1995, **68**:988-996.

55. Lieber AD, Yehudai-Resheff S, Barnhart EL, Theriot JA, Keren K: **Membrane tension in rapidly moving cells is determined by cytoskeletal forces**. *Curr Biol* 2013, **23**:1409-1417.

56. Morris CE, Homann U: **Cell surface area regulation and membrane tension**. *J Membr Biol* 2001, **179**:79-102.

57. Diz-Munoz A, Thurley K, Chintamen S, Altschuler SJ, Wu LF, Fletcher DA, Weiner OD: **Membrane tension acts through PLD2 and mTORC2 to limit actin network assembly during neutrophil migration**. *PLoS Biol* 2016, **14**:e1002474.

58. Paluch E, Heisenberg CP: **Biology and physics of cell shape changes in development**. *Curr Biol* 2009, **19**:R790-R799.

59. Yu H, Mouw JK, Weaver VM: **Forcing form and function: biomechanical regulation of tumor evolution**. *Trends Cell Biol* 2011, **21**:47-56.

60. Lieber AD, Schweitzer Y, Kozlov MM, Keren K: **Front-to-rear membrane tension gradient in rapidly moving cells**. *Biophys J* 2015, **108**:1599-1603.

61. Shi Z, Graber ZT, Baumgart T, Stone HA, Cohen AE: **Cell membranes resist flow**. *Cell* 2018, **175**:1769-1779 e1713.

62. • Parton RG: **Caveolae: structure, function, and relationship to disease**. *Annu Rev Cell Dev Biol* 2018, **34**:111-136.
An excellent, recent review illustrating through clinical examples how, by caching plasma membrane, caveolae protect cells/tissues from mechanical stress.

63. Petty HR: **Frontiers of complex disease mechanisms: membrane surface tension may link genotype to phenotype in glaucoma**. *Front Cell Dev Biol* 2018, **6**:32.

64. Wullschleger S, Loewith R, Hall MN: **TOR signaling in growth and metabolism**. *Cell* 2006, **124**:471-484.

65. • Berchtold D, Piccolis M, Chiaruttini N, Riezman I, Riezman H, Roux A, Walther TC, Loewith R: **Plasma membrane stress induces relocalization of Slm proteins and activation of TORC2 to promote sphingolipid synthesis**. *Nat Cell Biol* 2012, **14**:542-547.
First demonstration that TORC2 activity is regulated specifically by changes in plasma membrane tension.

66. •• Riggi M, Niewola-Staszkowska K, Chiaruttini N, Colom A, Kusmider B, Mercier V, Soleimanpour S, Stahl M, Matile S, Roux A *et al.*: **Decrease in plasma membrane tension triggers PtdIns (4,5)P2 phase separation to inactivate TORC2**. *Nat Cell Biol* 2018, **20**:1043-1051.
This work demonstrates that a membrane can be the relevant target of a drug-like small molecule.

67. •• Colom A, Derivery E, Soleimanpour S, Tomba C, Molin MD, Sakai N, Gonzalez-Gaitan M, Matile S, Roux A: **A fluorescent membrane tension probe**. *Nat Chem* 2018, **10**:1118-1125.
The first in class fluorescent probe to detect membrane tension is calibrated *in vitro* and applied *in vivo* to measure membrane tension.

68. Eger EI 2nd, Raines DE, Shafer SL, Hemmings HC Jr, Sonner JM: **Is a new paradigm needed to explain how inhaled anesthetics produce immobility?** *Anesth Analg* 2008, **107**:832-848.

69. Pickholz M, Oliveira ON Jr, Skaf MS: **Interactions of chlorpromazine with phospholipid monolayers: effects of the ionization state of the drug**. *Biophys Chem* 2007, **125**:425-434.

70. Franks NP, Lieb WR: **Molecular mechanisms of general anaesthesia**. *Nature* 1982, **300**:487-493.

71. Herold KF, Sanford RL, Lee W, Andersen OS, Hemmings HC Jr: **Clinical concentrations of chemically diverse general anesthetics minimally affect lipid bilayer properties**. *Proc Natl Acad Sci U S A* 2017, **114**:3109-3114.

72. He C, Hu X, Weston TA, Jung RS, Sandhu J, Huang S, Heizer P, Kim J, Ellison R, Xu J *et al.*: **Macrophages release plasma membrane-derived particles rich in accessible cholesterol**. *Proc Natl Acad Sci U S A* 2018, **115**:E8499-E8508.