

# Membrane-mediated Activity of Local Anesthetics

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## Abstract

The activity of local anesthetics (LAs) has been attributed to the inhibition of ion channels, causing anesthesia. However, there is a growing body of research showing that LAs act on a wide range of receptors and channel proteins, far beyond simple analgesia. The current concept of ligand recognition may no longer explain the multitude of protein targets influenced by LAs.

We hypothesize that LAs can cause anesthesia without directly binding to the receptor proteins, just by changing the physical properties of the lipid bilayer surrounding these proteins and ion channels, based on LAs' amphiphilicity. It is possible that LAs act in one of the following ways: they (a) dissolve raft-like membrane micro-domains, (b) impede nerve impulse propagation by lowering the lipid phase transition temperature, or (c) modulate the lateral pressure profile of the lipid bilayer. This could also explain the numerous additional effects of LA besides anesthesia.

Furthermore, the concepts of membrane-mediated activity and binding to ion channels do not have to exclude each other. If we were to consider LA as the middle part of a continuum, between unspecific membrane mediated activity on one end, and highly specific ligand binding on the other end, we could describe LA as the link between the unspecific action of general anesthetics, and toxins with their highly specific receptor binding.

This comprehensive membrane-mediated model offers a fresh perspective to clinical and pharmaceutical research and therapeutic applications of local anesthetics.

## Statement of significance

Local anesthetics, according to the WHO, belong to the most important drugs available to mankind. Their ***re-discovery as therapeutics, not only anesthetics***, marks a milestone in global pain therapy. The membrane-mediated mechanism of action proposed in this review can explain their puzzling variety of target proteins and their thus far inexplicable therapeutic effects. The new concept presented here places LAs on a continuum of structures and molecular mechanisms in-between small general anesthetics and the more complex molecular toxins.

## 1. Diversity of molecular targets of local anesthetics

The mechanism underlying the activity of local anesthetics (LAs) has appeared to be clear since the early 1950s (Fleckenstein 1950). In this classic picture their anesthetic effect is based on the inhibition of voltage-gated ion channels (VGC) (Butterworth and Strichartz 1990), especially the sodium ion channel (Catterall 2017). After passing through the cell membrane, LAs are thought to interact with a specific binding site inside the lumen, which they reach via a cytosolic channel entrance. This way, they induce a conformational change, rendering the pore impermeable to sodium ions. The depolarization caused by this blockage impedes impulse propagation in excitable cells, such as nerve and muscle cells.

Since 2000, a multitude of new molecular targets for LAs have been reported, even for one and the same LA molecule. Most of the targets are integral membrane proteins, some are present at the membrane surface, some are non-channel proteins, and even intracellular soluble proteins can be influenced by LAs (Table 1) (Tsuchiya and Mizogami 2013, Lirk, Picardi et al. 2014).

These new findings coincided with further reports on a variety of different clinical effects of LAs, such as anti-inflammatory (Hollmann, Durieux et al. 2001, Cassuto, Sinclair et al. 2006), anti-thrombotic (Lo, Honemann et al. 2001), or immunomodulatory (Cassuto, Sinclair et al. 2006) activities. These LA features were referred to as “alternative effects” (Pecher, Bottiger et al. 2004). Obviously, LAs possess a much broader spectrum of clinically important properties, extending far beyond their pure analgesic action. LAs are therefore recognized to play an increasingly important role as therapeutics (Weinschenk 2012). These pleomorphic effects of LAs may be useful in the treatment of chronic pain, chronic inflammation, chronic functional disorders (Weinschenk 2020), and even prevention of cancer recurrence (Grandhi and Perona 2019).

## 2. Diversity of specific binding sites?

Two striking observations are inconsistent with the conventional model of specific binding: (a) the multitude of target proteins for one and the same molecule (see Table 1), and (b) the structural diversity of LAs themselves (Tsuchiya and Mizogami 2013, Lirk, Picardi et al. 2014) that can nonetheless act on the same target protein. Considering this diversity and pleiotropy it is no longer plausible to assume that there exist so many different specific

receptor-ligand interactions, and to connect the different types of LAs with various specific targets. Each LA would have to match binding sites in many target proteins, and each target would have to possess specific binding pockets for a wide range of LAs. In this review, we discuss the hypothesis that an indirect, membrane-mediated activity provides a better explanation of the broad spectrum of LA action.

There is a common denominator of LAs: They all possess a distinctly amphiphilic structure, allowing them to position themselves within the amphiphilic region of a lipid bilayer. LAs typically consist of a hydrophobic aromatic moiety and a polar protonable amine segment, which are linked either by an amide or an ester bridge (**Error! Reference source not found.**). This amphiphilicity and membrane solubility is essential for their action as well as their pharmacokinetics: Depending on the pKa value, a sizable fraction of LAs possess an unprotonated amine and can therefore insert deeply into the polar/ apolar interface of a lipid bilayer. The depth of insertion into the bilayer depends not only on the pKa, but also on the relative size of the hydrophobic and hydrophilic segments. These intrinsic properties have fundamental implications for the activity of LAs even in the classical model.

According to the classical picture of LA activity, the molecules are endowed with a certain level of hydrophobicity allowing them to pass right through the membrane to reach the lumen of the ion channel via the cytosolic side. This raises the question, however, whether they really need to traverse the membrane to reach their target, or whether they might rather target the lipid bilayer itself and act laterally while residing within the membrane?

In this review we discuss three alternative ways to explain LA activity by such membrane-mediated mechanisms (Figure 2), which will be described in detail in section 4:

(1) **Lipid rafts:** Rafts are local lipid micro-domains with a defined lipid composition, which exist in a so-called liquid-ordered phase. They can recruit and cluster specific membrane proteins as part of their functional cycle due to the increased bilayer thickness and reduced lipid dynamics. LAs can disperse these domains, thereby affecting the activity of the raft-associated proteins (Figure 2A).

(2) **Nerve impulse propagation by solitons:** Nerve impulses have been suggested to be formed by sound waves with special characteristics (“solitons”). These can travel laterally through the lipid bilayer without fading out, provided that the bilayer is close to its phase

transition temperature and thereby possesses particular physical properties. In this theory, nerve impulses do not rely on the opening and closing of ion channels. The change in lipid phase transition temperature after insertion of LAs in membranes may eradicate the conditions necessary for wave propagation (Figure 2B).

(3) **Lateral pressure profile:** It is well known that membrane protein function can be influenced by the lateral pressure profile (LPP) in the lipid bilayer (Cantor 1999, Marsh 2007). Here, we propose that the presence of LAs in the membrane will modulate the LPP and thereby affect the functions of numerous types of membrane proteins (Figure 2C).

### 3. Membrane Effects of Local Anesthetics

In the 1980s, the influence of LAs on the physical properties of lipid bilayers was demonstrated by fluorescence polarization and solid-state NMR (Boulanger, Schreier et al. 1981, Seelig, Allegrini et al. 1988, Yun, Cho et al. 2002, Kinoshita, Chitose et al. 2019). LAs bind to membranes in a pH-dependent manner: When protonated at low pH (i.e. in their charged form) they are localized near the headgroup region of a lipid bilayer, whereas uncharged, they penetrate deeper into the membrane, below the glycerol backbone (Boulanger, Schreier et al. 1981, Kelusky, Boulanger et al. 1986, Weizenmann, Huster et al. 2012).

LAs are also known to decrease the bilayer thickness (Turner and Oldfield 1979), and to increase fluidity in the hydrophobic membrane core while lowering it in the head group region (Yun, Cho et al. 2002). The localization of LAs in or just below the headgroup region was determined by solid-state  $^2\text{H}$ -NMR, based on changes in the order parameter and mobility of individual lipid segments (Boulanger, Schreier et al. 1981, Seelig, Allegrini et al. 1988). When immersed in the headgroup region, LAs require additional space and increase the lateral volume of the bilayer there. Such changes in the elastic and structural properties are accompanied by a modulation of the electrostatic properties, as LAs have been found to alter the electric dipole and surface charge (Seelig, Allegrini et al. 1988, Castro, Stevensson et al. 2008).

Physicochemical effects of LA on the membrane have been found in LA concentrations of about 0.1 LA molecules per lipid molecules (Boulanger, Schreier et al. 1981, Seelig, Allegrini et al. 1988, Castro, Svensson et al. 2008). What are the LA concentrations in clinical applications, and are these concentrations comparable to those used in model membrane setups needed for the physicochemical studies? A typical dosage of LA is about 50-100 mg, which in an approx. 50 ml, tissue volume would result in a local concentration of ~ 10 mM. There are only a few quantitative studies on the partition coefficient of LA in lipid membranes, where values between  $\sim 10 \text{ M}^{-1}$  and  $\sim 10^3 \text{ M}^{-1}$  (mol LA per mol lipid per LA in water in  $\text{M}^{-1}$ ) have been found, depending strongly on pH, ionic strength, and lipid type (Kelusky, Boulanger et al. 1986, Seelig, Allegrini et al. 1988). Assuming a local anesthetic concentration of ~ 10 mM, these partition coefficients would result into 0.1 - 10 LA molecule per lipid molecules. 0.1 molecules per lipid has been estimated for the partitioning of general anesthetics (Herold, Sanford et al. 2017). Therefore, the concentrations in vitro are comparable to those used in clinical application in vivo.

#### 4. Mechanisms of membrane-mediated activity of local anesthetics

The local lipid environment plays a critical role in the function of membrane proteins (Nyholm, Ozdirekcan et al. 2007, Phillips, Ursell et al. 2009, Zhou and Cross 2013). Given that the presence of LAs affects the physicochemical properties of lipid bilayers – how can this modulation be translated into a change in membrane protein function?

##### (1) LAs disperse lipid rafts:

Lipid micro-domains, or rafts, are a controversially discussed concept. Rafts represent areas in cell membranes which recruit receptors and other proteins to form functionally important protein-rich patches (Hooper 1998, Simons and Toomre 2000, Lichtenberg, Goñi et al. 2005). They consist mainly of sphingolipid and cholesterol, which segregate from the remaining lipids. Due to their tight packing, the sphingolipid/cholesterol mixture forms a more viscous “liquid ordered” ( $L_o$ ) phase with solubility properties different from the remaining lipid bilayer. Small guest molecules in the membrane, as well as membrane proteins, show a

preference for either the lipid raft domain or the surrounding fluid membrane. Receptors like NMDA, GABA-receptor (Besshoh, Bawa et al. 2005, Li, Serwanski et al. 2007, Sierra-Valdez, Ruiz-Suarez et al. 2016), and ion channels involved in nerve impulse propagation (O'Connell, Martens et al. 2004, Maguy, Hebert et al. 2006, Balijepalli and Kamp 2008, Dart 2010, Amsalem, Poilbout et al. 2018), can accumulate in lipid rafts, which may be crucial for their activity-(Besshoh, Bawa et al. 2005, Maguy, Hebert et al. 2006, Li, Serwanski et al. 2007, Dart 2010).

LAs interact with such micro-domains (Tsuchiya, Ueno et al. 2010, Bandejas, Serro et al. 2013, Yoshida, Takashima et al. 2015, Kinoshita, Chitose et al. 2019). They decrease the lipid order and increase the fluidity in raft-like domains, eventually dispersing and dissolving them. The strength of this effect on rafts has been reported to correlate with the depth at which the LA molecules are inserted into the membrane and depend on their bulkiness. For example, Kinoshita et al., using small angle X-ray scattering and fluorescence anisotropy, found that dibucaine, tetracaine and lidocaine, in this order, eliminate lipid phase segregation (Kinoshita, Chitose et al. 2019). This physical effect correlates with the ranking in which they decrease lipid order and insert more deeply into the membrane (Kinoshita, Chitose et al. 2019).

However, it is not clear whether there is a preferential interaction of LAs with lipid rafts compared to other lipid mixtures, and so the relevance of their interaction with raft-like domains remains unclear (Tsuchiya, Ueno et al. 2010). Tsuchiya et al. showed that the lipid disordering effect of LAs was weaker on raft lipid mixtures than on biomimetic lipid mixtures of phosphatidylcholine, phosphatidylethanolamine, and cholesterol (Tsuchiya, Ueno et al. 2010, Tsuchiya and Mizogami 2013). They found a strong correlation between the lipid disordering caused by LAs and their pharmacological activity in the case of non-raft lipid compositions, although to a much lower extent in the case of raft-forming lipids.

Nonetheless, even if the disordering by LAs is weaker in raft-forming lipids, it may still affect the sequestered membrane proteins when they are released upon dispersal of the rafts — at least when compared to other proteins that normally reside in the usual fluid lipid phases.

A weakening or dissolution of raft-like domains by LAs has indeed been shown to influence the concentration and activity of receptors or ion channels enriched in these domains. For example, LAs reduce the concentration of NMDA and GABA receptors in raft-like domains in

brain lipids (Sierra-Valdez, Ruiz-Suarez et al. 2016). The interference with rafts also plays a role in the inhibition of malaria infection by lidocaine (Kamata, Manno et al. 2008, Koshino and Takakuwa 2009). Lidocaine, applied to erythrocytes, effectively prevents parasite invasion by inhibiting Gs $\alpha$ -mediated signal transduction involving GPCRs that are associated with raft-like micro-domains (Kamata, Manno et al. 2008). An accumulation in raft-like domains has been discussed for a wide range of receptors and ion channels, hence the idea that LAs act indirectly by influencing these domains could explain their broad spectrum of activities.

Is this mechanism in agreement with the established facts of local anesthetic action? Does it explain (a) LA effects on voltage-gated sodium channels involved in nerve impulse propagation, and (b) the wide range of “alternative” effects involving many membrane protein receptors or channels? Preliminary data show that voltage-gated sodium channels may be associated with rafts, and moreover, their function could be impeded when this raft-like lipid environment is destroyed (Maguy, Hebert et al. 2006, Balijepalli and Kamp 2008, Dart 2010, Pristera, Baker et al. 2012, Amsalem, Poilbout et al. 2018). A raft-related action of LAs could thus have an impact on the relevant sodium channels. Since micro-domain formation plays an important role for the function of other membrane proteins as well, a mechanism involving rafts could also explain the broad spectrum of LA activities. However, it is questionable whether all those “alternative” effects ascribed to LAs involve membrane proteins that are segregated into rafts, or whether some other models of explaining a membrane-mediated activity would impose fewer constraints.

## **(2) LAs Impede Soliton Propagation:**

Another theory for the activity of LAs related to their influence on membrane fluidity was proposed by Heimburg in 2005. He suggested a model for nerve impulse propagation based on soliton waves (Heimburg and Jackson 2005). Solitons are wave packages that can propagate without broadening as they travel, hence without fading out or dissipating. This special form of waves can only occur in media such as lipid bilayers, which have both a non-linear and dispersive character. Under certain conditions the broadening due to the frequency-dependent velocity of a dispersive wave can be compensated for by the



frequency-dependent amplitude – then the wave can propagate without changing its shape as long as it travels.

Lipid bilayers possess these necessary properties for soliton-like sound waves at temperatures near their gel-fluid phase transition (Heimburg and Jackson 2005). Near phase transitions, thermodynamic properties such as compressibility or heat capacities diverge and endow the lipid membrane with the non-linearity and dispersion required for solitons. As such, a nerve impulse could be, according to Heimburg, a high-density patch of lipids travelling as a soliton-like sound wave along the axon. The existence of such solitons has been shown, but the concept still needs further corroboration (Shrivastava and Schneider 2014, Gonzalez-Perez, Mosgaard et al. 2016) from other groups.

Heimburg suggests that – within the soliton model – the activity of LAs may indeed be explained by their influence on membrane fluidity. It is well-known that LAs lower the phase transition temperature of a lipid bilayer when inserted, like the freezing point depression of water by solutes (Lautrup, Appali et al. 2011). Consequently, LAs shift the phase state of membranes towards the fluid phase. This would then no longer be favorable for the formation of solitons, impairing the propagation of nerve impulses. Biological membranes have rather broad phase transitions around ambient temperature. Hence, an inhibition by LAs is possible. However, it is unclear whether LA would be able to shift the membrane system sufficiently away from conditions required for solitons, given that the phase transitions of natural membranes are rather broad. If they are, this membrane-mediated model does not require any specific binding of LAs to post-synaptic ion channels and could therefore explain their analgesic action.

Is this mechanism able to explain (a) LA effects on voltage-gated sodium channels involved in nerve impulse propagation, and (b) the wide range of “alternative” effects involving many membrane protein receptors or channels? Within the framework of nerve impulse propagation through solitons, voltage gated sodium channels would not be involved, or at least not play an exclusive role. Here the activity of LAs is based on their ability to lower the phase transition temperature of the lipid membranes, and thereby impede the conditions for soliton formation. This change in the properties of the membranes may also affect membrane receptors and channels involved in the “alternative effects” of LAs, or even cause those activities without involving any proteins at all. However, it remains unclear whether

the change of a single variable, the phase transition temperature, could explain the various complex responses to LAs. Additionally, the influence of LAs on other targets not connected to nerve propagation is difficult to explain within the soliton theory.

### **(3) LA Modulate the Lateral Pressure Profile:**

Because LAs are amphiphilic molecules, they become embedded in the lipid bilayer and modulate the physical properties of the membrane. Ion channels and other membrane proteins could adapt to such changes in the environment, resulting in an indirect membrane-mediated effect of LAs.

The lateral pressure profile (LPP) has been demonstrated as a major physical property of bilayers that can modulate the structure and function of membrane proteins (Cantor 1999, Marsh 2007, Mouritsen 2011). Surface tension arises when lipids form a bilayer that is interfaced between water. This tension is balanced by repulsive forces acting laterally within the plane of the bilayer, the so-called lateral pressure. This pressure is not equally distributed across the membrane but follows a distinct profile (Error! Reference source not found.): It has a positive maximum in the headgroup region and in the acyl chain region (representing lateral “pressure”), and a sharp negative peak near the ester bonds of the glycerol backbone, representing the attractive force due to the separation of hydrophobic lipids from water. The pressures in the bilayer can reach remarkably high values. A typical surface tension is ~50 mN/m. As this tension is acting over less than the ~5 nm membrane thickness, the resulting pressure can reach up to ~1000 atm (Cantor 1999).

The LPP model can be seen as a generalization of the idea of “spontaneous curvature” of membranes (Marsh 2007). The most favorable geometry of a single monolayer leaflet of the membrane can deviate from a planar slab by being curved, due to differential cross-sectional demands of the headgroup and acyl chain regions of the lipid molecules. Despite this intrinsic curvature, the monolayer is forced into a planar slab when the bilayer is assembled from the two opposing monolayers. Hence, a curvature frustration arises which results in lateral forces, as described by the LPP model. The lateral pressure profile is known to influence membrane proteins and peptides. For example, they may have different

propensities for inserting themselves into a lipid bilayer depending on how well their cross-sectional shape profile is adapted to the LPP. The membrane insertion of peptides such as alamethicin, or enzymes such as CTP:phosphocholine cytidylyltransferase, depend on lipid spontaneous curvature (Marsh 2007).

Which conformational state of a protein is energetically favored will depend on how well the actual shape of the protein matches the LPP. For example, the equilibrium between the meta-I and meta-II states of rhodopsin depends on the spontaneous curvature of the lipid environment (Botelho, Gibson et al. 2002). Furthermore, bacterial mechanosensitive channels, as well as eukaryotic channels such as TREK-1, TREK-2, TRAAK, TRP channels, or PIEZO, are activated by a “force-of-lipids” (Martinac, Adler et al. 1990, Corey 2003, Berrier, Pozza et al. 2013, Lolicato, Riegelhaupt et al. 2014, Battle, Ridone et al. 2015, Aryal, Jarerattanachai et al. 2017, Cox, Bavi et al. 2017, Ridone, Grage et al. 2018). The bacterial mechanosensitive channel proteins MscL and MscS belong to a protein system that regulates osmotic pressure and swelling of the cell. They open when the mechanical tension in the lipid bilayer exceeds a particular threshold value (Martinac, Buechner et al. 1987, Martinac, Adler et al. 1990, Sukharev, Blount et al. 1994, Perozo, Cortes et al. 2002). Shifts in the LPP and membrane thickness serve as triggers to open these mechanosensitive channels (Martinac, Adler et al. 1990, Gullingsrud and Schulten 2004, Jeon and Voth 2008, Ollila, Risselada et al. 2009, Grage, Keleshian et al. 2011, Ollila, Louhivuori et al. 2011, Battle, Ridone et al. 2015, Ridone, Grage et al. 2018). Interestingly, an asymmetric change of the LPP seems to be important to achieve complete opening of MscL (Martinac, Adler et al. 1990, Perozo, Kloda et al. 2002).

A change in the LPP can arise from varying lipid composition and changing the spontaneous curvature of the constituents. In bacterial mechanosensitive channels, for example, increasing desaturation of the lipid chains can redistribute the lateral pressure within the bilayer towards the center of the membrane, which triggers changes in membrane protein activity (Carrillo-Tripp and Feller 2005, Ridone, Grage et al. 2018). Even molecules other than lipids, which associate with membranes, can exert a lateral force at a certain depth where they are accommodated in the membrane (Perozo, Kloda et al. 2002, Jerabek, Pabst et al. 2010, Fabian, Sega et al. 2017). Consequently, their immersion could alter membrane protein conformation and function, as has already been proposed in the 1950s by the so-

called critical volume theory. According to this theory, drugs that act non-specifically will become toxic when their volume fraction in the lipid bilayer exceeds a critical value, which is accompanied by swelling of the membrane (Mullins 1954).

LAs are usually administered at such high local concentrations that it is highly likely they also exert a lateral force when bound to the lipid bilayer. Right at the level of their localization they will alter the lateral pressure profile and influence the conformation of the surrounding membrane proteins. There are few experimental studies so far, but membrane-mediated effects have been indeed observed, e.g., for bacterial mechanosensitive channels (Martinac, Adler et al. 1990). Procaine and tetracaine, amongst other amphiphiles, have been reported to lower the opening threshold of bacterial mechanosensitive channels when added asymmetrically to one of the two leaflets of a lipid bilayer (Martinac, Adler et al. 1990, Martinac, Adler et al. 1990).

How well can this third model, which attributes the activity of LAs to changes in the LPP, account for the important properties of LA action? Does it explain the known interaction of LAs with sodium channels, and could it also rationalize the alternative effects of LAs? Regarding the role of an LPP-mediated action of LAs to voltage gated sodium channels, it is not known whether these proteins are influenced by lateral pressure in the membrane or not. Nonetheless, they are certainly susceptible to the membrane environment, as shown by their association to raft-like domains (O'Connell, Martens et al. 2004, Amsalem, Poilbout et al. 2018). The sensitivity of sodium channels to the lipid environment has also been deduced from the influence of free fatty acids on the gating behavior and toxin binding of voltage gated sodium channels (Kang and Leaf 1996, Wieland, Gong et al. 1996, Bendahhou, Cummins et al. 1997). Interestingly, the impact of free acids depends on the degree of their unsaturation. The largest inhibition of toxin binding or modulation of channel activity has been achieved with poly-unsaturated fatty acids. This finding was interpreted by the authors as specific binding of the fatty acids to the channel protein. However, this observation agrees just as well with an indirect modulation via the LPP, which has been described for poly-unsaturated lipids (Carrillo-Tripp and Feller 2005, Ridone, Grage et al. 2018). Therefore, it seems plausible that ion channels related to nerve impulse propagation should be sensitive to the LPP.

A modulation of the LPP by LAs may also explain the enormous range of alternative LA effects. Since the lateral pressure varies across the membrane, and different LAs affect the LPP in different ways depending on their affinity and penetration depth into the membrane, we can expect a wide range of membrane-mediated responses of membrane proteins to LAs. Hence, a diverse spectrum of widely varying activities of LA should arise from such a LPP-mediated mechanism, allowing the LPP model to easily explain the various alternative LA effects.

## 5. LAs: membrane-mediated action *versus* specific binding site?

Irrespective of the details of a membrane-mediated mechanism of LAs, the question needs to be addressed as to how this general concept compares with the classical picture of specific binding sites for LAs in voltage gated sodium channels.

A counter argument to the membrane-mediated action of both general and local anesthetics is that the concentrations needed to modulate membrane properties exceed those used in clinical applications. When Herold et. al used an indirect assay based on gramicidin A conductance to measure the membrane thinning induced by general anesthetics (GAs), they found no significant effects at mole fractions (per lipid) of 0.01 to 0.1, which correspond to values required for anesthesia (Herold, Sanford et al. 2017). Furthermore, when LA-induced membrane thinning was eventually observed from  $^2\text{H}$ -NMR order parameters, it required fairly high mole fractions, significantly above 0.1, i.e. well above clinical usage (Turner and Oldfield 1979, Culetto 2019). Nonetheless, it may be premature to rule out a membrane-mediated effect solely based on membrane thickness. Small shifts in the detected physical parameters can indicate larger changes of other physical properties that are relevant for the interaction with membrane proteins. For example, a modulation of the lateral pressure profile may be accompanied by only a minor adjustment of the overall membrane thickness. Furthermore, the observation of a cutoff limit in the anesthetic effect of n-alkanols has been used to argue in favor of specific interactions with membrane proteins. N-alkanols show activity loss if the chain length is extended beyond a threshold length. This cutoff effect has been attributed to the finite size of specific binding pockets, assuming a specific interaction of the anesthetic with the membrane proteins. However, a membrane-mediated mechanism

is just as compatible with such a cutoff because the change in lateral pressure will depend critically on the insertion depth, hence on the length of the n-alkanol (Eckenhoff, Tanner et al. 1999, Mohr, Gribble et al. 2005).

Moreover, for the several LAs that exist in two chiral forms, the two enantiomers often exhibit different anesthetic potencies, as would be expected for a specific interaction with a binding site on a protein (Mizogami, Tsuchiya et al. 2008, Tsuchiya and Mizogami 2013). However, the difference is never all-or-nothing, and the activities often vary only by a few percent. This moderate stereospecificity may thus be better explained by the inherent chirality of the other membrane constituents. Namely, cholesterol is chiral, which is abundant in most eukaryotic membranes, particularly in raft-like domains, and could lead to different interactions of stereoisomeric LAs with the lipid bilayer. Phospho- and glycolipids, too, are chiral at the central position of the glycerol backbone.

In the light of ample experimental evidence (which will not be reviewed here), it is clear that the specific binding of LAs to ion channels involved in nerve impulse propagation and their direct interaction with the protein surface cannot be denied when considering the anesthetic action of LAs. However, these data do not rule out the possibility that LAs are able to act in both ways, (a) via direct interaction with the ion channel, as well as (b) through a membrane-mediated mechanism. It is interesting to note that there are two binding sites located inside the sodium channels: One site, near the fenestrations, can be reached from the lipidic environment and is related to tonic block of the channel. Another site, located near the activation gate, can be reached through the cytosolic channel entrance and is considered to be related to use-dependent block (Boiteux, Vorobyov et al. 2014, Martin and Corry 2014, Clairfeuille, Xu et al. 2017, Buyan, Sun et al. 2018).

This commonly-known fact points towards the possibility of further, less-specific binding sites, as they have been observed for instance in the binding of the general anesthetic propofol to the prokaryotic Na<sub>v</sub> channel NaChBac (Wang, Yang et al. 2018). A total of six sites on each of the four subunits were predicted by MD simulations, four of which could be confirmed experimentally by NMR. One of these low-affinity sites was shown to overlap with a binding site for other LAs, suggesting that there may exist a continuum of affinities and preferential interactions between local (LAs) as well as general anesthetics (GAs) and membrane proteins.

In fact, we obtain a broader view when comparing LAs with GAs, which may be considered prototypes for membrane-mediated action, as well as with toxins, which are prototypes for high-affinity interactions at specific binding sites. Given their hydrophobic portion, LAs carry the typical core structural features of GAs, which are responsible for a membrane-mediated activity. At the same time, LAs also possess additional structural properties (steric variability, polarity, H-bonding, charge) endowing them with enough structural diversity to discriminate between binding sites on proteins. This way, in a ranking of increasing specificity, LAs would stand between entirely non-specific GAs and those highly specifically binding compounds like toxins, as illustrated in figure 4.

## 6. General anesthetics, local anesthetics, toxins – a continuum?

General anesthetics (GAs) were amongst the first molecules for which a membrane-mediated mechanism has been proposed (Cantor 1997). These hydrophobic molecules partition well into the lipid bilayer, where they are localized either near the core of the bilayer, or below the carbonyl region in the upper acyl chain region (Fabian, Segal et al. 2017). GAs modulate the physical properties of the membrane and thereby influence the equilibrium between different conformational states of the affected proteins, which is apparent from the response in protein kinetics (Cantor 2001). GAs typically have a very hydrophobic nature and dissolve well in the bilayer but lack the structural diversity to be able to recognize specific binding sites; hence, they are generally understood to act indirectly via the membrane (Figure 4A).

LAs possess an amphiphilic structure composed of two parts: (a) a hydrophobic segment, which drives its binding to the lipid bilayer and thereby facilitates a membrane-mediated action, and (b) a polar segment that gives the molecule a bipolar characteristic, which allows binding to protein surfaces at patches matching this amphiphilic pattern. Hence, we may consider LAs to be a structurally expanded version of GAs that are able to act through the same underlying membrane-mediated mechanism, but additionally bear a more specific ability to block ion channels and possibly other membrane proteins (Figure 4B).

It is not surprising that LAs are able to bind some structural proteins and water-soluble enzymes, like the ones listed in Table 1, if they carry a suitable amphiphilic patch on their

surface. In terms of mass action and local concentration, however, the effect on membrane proteins will dominate by far.

The interaction of LAs with ion channels may be regarded as rather indiscriminate, when comparing the affinity to patches of similar amphiphilic characteristics with the classical key-lock or hand-glove recognition mechanisms known for toxins (Martin and Corry 2014). Indeed, for the putative binding sites in the core of the channel which are responsible for resting or flicker block, only low affinities in the mM range and binding through hydrophobic interactions have been reported (Fozzard, Sheets et al. 2011). Whether the reported high-affinity binding of the use-dependent block is associated with a highly specific binding pocket is not yet fully understood.

Any specific binding requires a certain complexity in the molecular structure of the ligand, and an assortment of charged, polar, H-bonding, and hydrophobic patches, in order to allow the recognition of a binding site by many point-to-point interactions. Toxins are an excellent example for such highly specific interactions. Their complex, often peptide-like structure allows them to recognize specific target proteins and act even at nanomolar concentrations. At the same time, most toxins are water soluble, hence they do not possess the ability to act in a membrane-mediated way like general anesthetics (Figure 4C). Thus, in a more general view, GA, LA and toxins form a sequence of increasing complexity and decreasing hydrophobicity, resulting in a continuum of abilities to act through indirect influence on the membrane up to very specific protein binding (figure 5).

An intriguing position in this sequence of increasing molecular complexity is taken by the two well-known compounds propofol and cocaine. As already implied above, the GA propofol is closely related to LAs, given its moderately amphiphilic structure and promiscuous action. It has a fast-anesthetic impact of short duration that likely involves some membrane-mediated effects. It is also known to serve as an allosteric modulator of pentameric ion channels (GABA<sub>A</sub> and nicotinic acetylcholine receptors), and even produces psychotropic effects (Diaz and Kaye 2017). Our continuum model suggests that propofol should be positioned in between the purely hydrophobic GAs and the more complex amphiphilic LAs (Figure 5).



Cocaine, at the other end of our LA continuum, is the only known LA with a high toxicity. Cocaine is still a *-caine* LA, which blocks the voltage gated sodium channel. However, based on its rather complex molecular structure (steric form), it can also bind to a specific site on the dopamine transporter as well as other receptor proteins (Hearing, Zink et al. 2012). As an amphiphilic molecule, it fits well in the lateral membrane pressure model (Figure 5), and therefore takes an intermediate position between non-toxic LAs with their more pronounced membrane-mediated effects, and pure toxins with their specific ligand-binding site interactions. Although not all of their proposed effects are proven, in the future more information could provide novel insights about the role of lateral pressure in the action of local anesthetics.

## 7. Pharmacological and clinical implications and perspectives

In summary, our interpretation of LA action combines their spontaneous accumulation in the lipid bilayer and their membrane-mediated mechanism with an ability to target specific membrane proteins at moderate affinity. The inclusion of a membrane-mediated mechanism in the functional model could potentially clarify several phenomena concerning the action of LAs that have remained unexplained to date. Should further experimental data be provided, the following effects could be explained by the lateral pressure profile (LPP) hypothesis, which we regard as the most likely mode of membrane-mediated action:

**Targets and Pleiotropy.** The above-mentioned lateral membrane pressure model (LPP) can explain why LAs affect so many different target proteins. The LPP model predicts that there exist numerous membrane and non-membrane proteins that can be influenced by the very same LA molecule. It also explains why the same membrane protein can be affected by several different types of LAs.

**Duration and intensity.** The different duration and intensity of analgesic action of LAs may be largely attributed to their membrane immersion properties.

**Therapeutic relevance.** At the moment, the therapeutic effects of LAs are distributed into five groups (Weinschenk 2012): analgesia, anti-inflammation, perfusion enhancement, anti-thrombotic effects, and protection against cancer recurrence (Grandhi and Perona 2019).

This variety of therapeutic effects might be better explained by the LPP model than by classical protein-ligand interactions.

**Independence from structure.** It should be possible to predict the anesthetic effects of new LAs from their specific immersion characteristics in the cell membrane, regardless whether they are *-cains* or not.

**Blood-brain-barrier.** The LPP model could explain the different capability of LAs to cross the blood-brain-barrier by their membrane immersion.

**Addiction research.** The LPP model might explain why cocaine has central addictive effects not present in other *-cains*.

**New effects.** Further therapeutic effects can be predicted by extrapolating the influence of LAs to other membrane proteins, based on their physicochemical characteristics defined by the LPP model.

**New molecules.** Novel LAs could be designed with new characteristics by fine-tuning their affinity and localization in the lipid bilayer. The LPP model may offer new opportunities for drug discovery within or outside the pharmacological group of *-cains* and allow scientists to design new drugs with similar pharmacological features.

**Toxicity.** Some LAs are more toxic than others. The LPP model may attribute the therapeutic range to distinct their membrane affinity, depth of immersion, and structural complexity.

**LA Antidotes.** The LPP model may explain the ability of highly concentrated lipid suspensions to act as an antidote against toxic concentrations of LAs.

**Continuum from LAs to GA.** The LPP model helps to define a continuum between the mode of action of GAs, LAs and toxins. It implies a fundamentally related mode of action for LAs and GAs, that is extended by instilling further complexity into the LA structure.

**Universality.** Beyond LAs and GAs, the LPP model may represent a general mechanism of action for many small molecular drugs (which often obey the Lipinski rule-of-five). Based on their universal immersion into and transversal across cell membranes, some as yet unexplained effects might be understood, especially if the molecules are amphiphilic and administered at high concentration.

## 8. Conclusions

The lateral pressure profile model presented here, based on the amphiphilicity of membrane bound LAs, provides a fresh perspective to explain the quite different molecular effects of LA. It places LAs into a continuum between general anesthetics and toxins. This hypothesis should be pursued further by designated experimental assays. The LPP model may provide a multitude of new possibilities in the broad clinical use of LAs in therapy and analgesia.

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## 10. Author Contributions

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## 12. Footnotes

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## 13. Figure Legend

**Figure 1:** Amphiphilic structures of some representative local anesthetic and their predicted positions within the membrane. LAs possess typically consist of a hydrophobic aromatic moiety plus a polar protonable segment, linked by either an amide (Bupivacaine, Mepivacaine, Lidocaine, QX-314) or ester (Benzocaine, Procaine). They are characterized by different pKa values and octanol-water partition coefficients (both properties decreasing from left to right), leading to different degrees of partitioning into the membrane in a non-trivial order.

**Figure 2:** Different concepts for a membrane-mediated activity of local anesthetics. (A) LAs disperse the highly ordered lipid raft-like domains and thereby release the segregated membrane proteins. (B) LAs lower the phase

transition temperature and thereby impair the propagation of soliton-like nerve impulses. (C) LAs modulate the lateral pressure profile within the bilayer and thereby affect membrane protein function.

**Figure 3:** The lateral pressure profile of a lipid membrane. The attractive force due to the surface tension of the water-lipid interface (blue arrows) is balanced by repulsive lateral pressure in the headgroup regions and membrane core (red arrows). As a result, the lateral pressure  $p_z$  varies as a function of membrane depth  $z$  (right, schematic pressure profile).

**Figure 4:** Proposed continuum concept to illustrate the activities of general anesthetics, local anesthetics, and toxins in membranes. (A) General anesthetics possess simple non-descript hydrophobic structures that exhibit merely an indirect membrane-mediated activity. (B) Local anesthetics share their hydrophobic portion with general anesthetics, but contain a second, polar segment which allows specific localization within the bilayer membrane, as well as indiscriminate matching with suitable binding patches on proteins. (C) More complex molecular structures, such as toxins, interact precisely with highly specific binding sites on proteins.

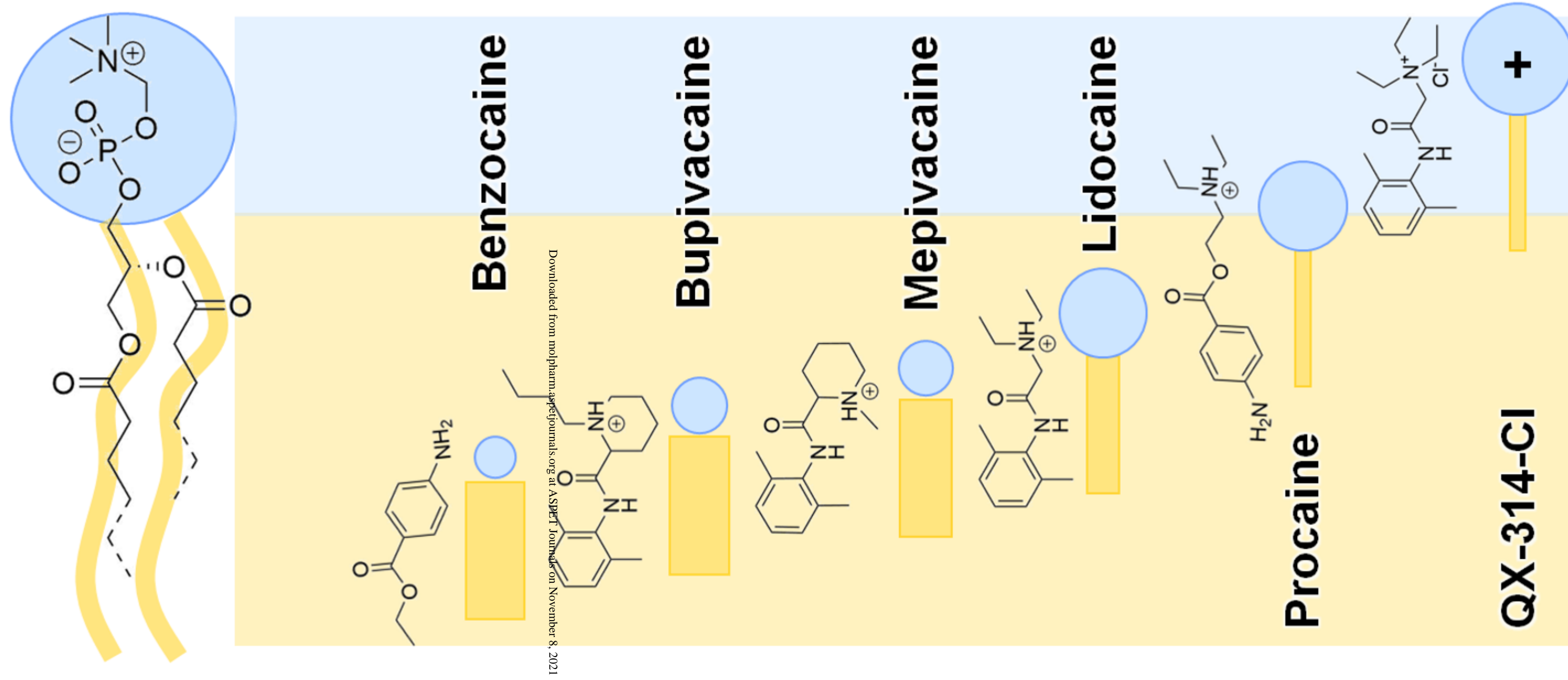
Figure 5: Special roles of propofol and cocaine. Propofol, known for its narcotic and hypnotic effect, achieves its activity through a more hydrophobic structure with low complexity, placing it in between general and local anesthetics. Cocaine exhibits a local anesthetic effect, but also a high toxicity which is due to a more complex structure allowing for specific interaction with protein targets. Many properties of cocaine can be explained by its position between local anesthetics and toxins. © images of Michael Jackson and Sigmund Freud with permission from 123RF Europe BV, NL-3447 GZ Woerden (M. Jackson, zinovskaya@123rf.de; S. Freud: [yetiyeaw@123rf.de](mailto:yetiyeaw@123rf.de)) from August 16<sup>th</sup>, 2021.

## 14. Tables

**Table 1: Target proteins of Local Anesthetics.**

Channels	Voltage gated Na <sup>+</sup> <sub>v</sub> channel (Butterworth and Strichartz 1990) K <sup>+</sup> channel (Guo, Castle et al. 1991) Ca <sup>2+</sup> channel (Xiong and Strichartz 1998) TRP 1-7 (Pareek, Keller et al. 2007, Leffler, Fischer et al. 2008, Puopolo, Binshtok et al. 2013)
Receptors	G <sub>αq</sub> proteins / GPCR (Hollmann, Wieczorek et al. 2001, Hollmann, McIntire et al. 2002, Benkwitz, Garrison et al. 2003) NMDA (Hahnenkamp, Durieux et al. 2006, Paganelli and Popescu 2015) m3 muscarinic AChR (Hollmann, Ritter et al. 2001) β <sub>2</sub> adrenergic receptor (Butterworth, James et al. 1997) GABA receptor (Sierra-Valdez, Ruiz-Suarez et al. 2016) glycine receptor (Hara and Sata 2007)
Enzymes	phospholipase (Hollmann, Gross et al. 2001) Na/K adenylate cyclase ATPase (Butterworth, Brownlow et al. 1993)
Structural molecules	microtubule based kinesin (Miyamoto, Muto et al. 2000)
Other targets	DNA demethylation (Stresemann, Brueckner et al. 2006, Tada, Imazeki et al. 2007)

Figure 1



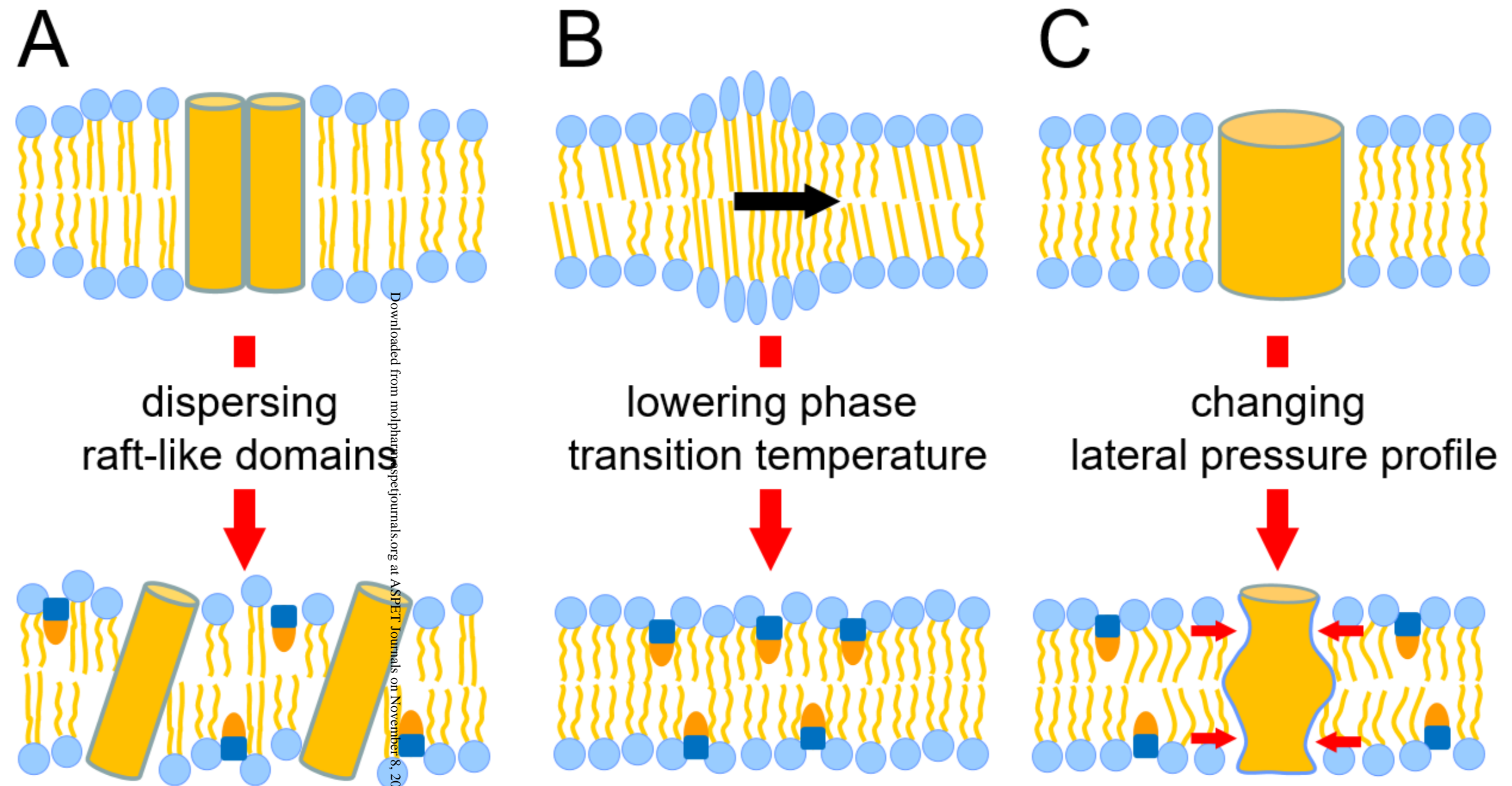


Figure 2

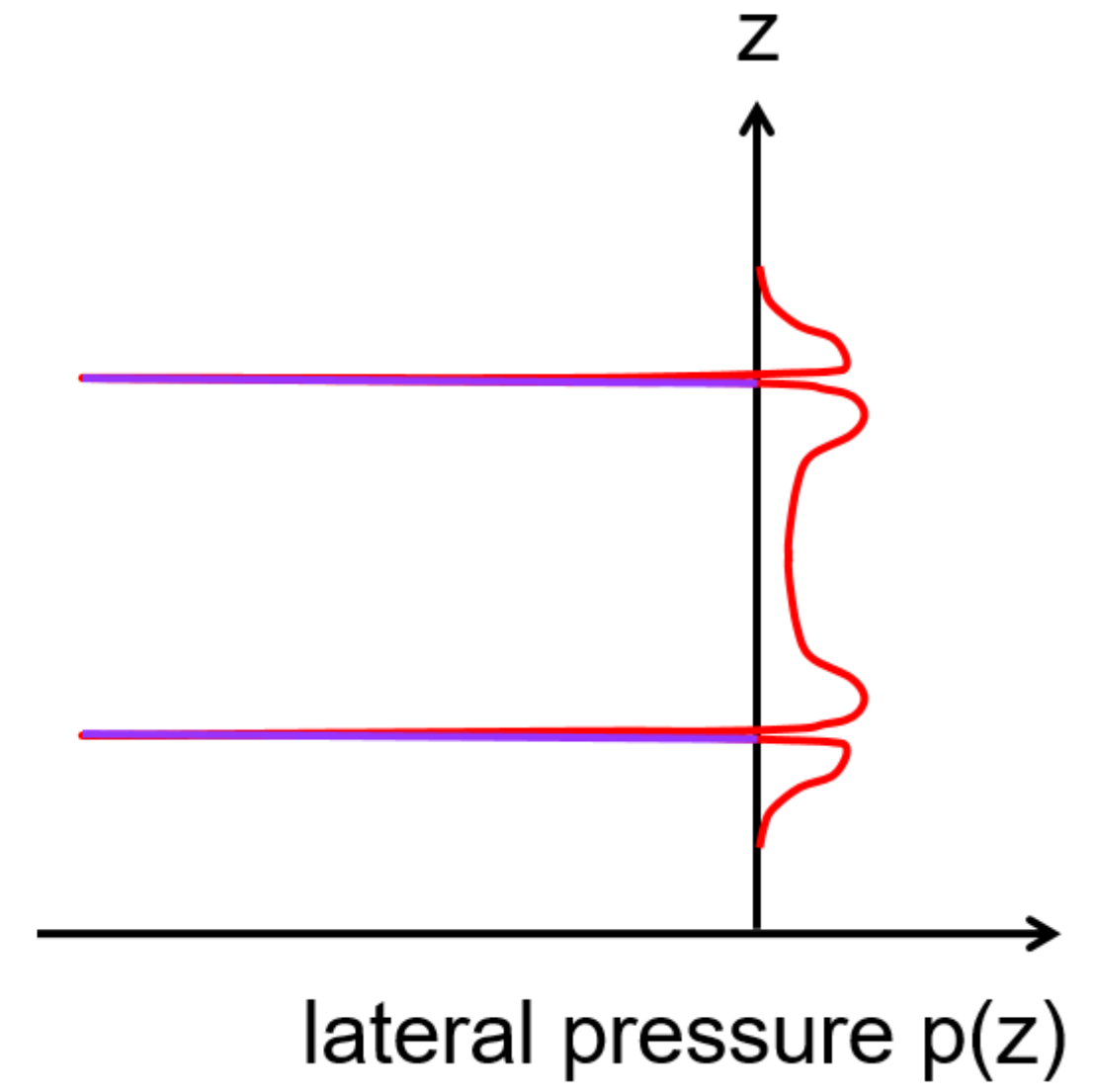
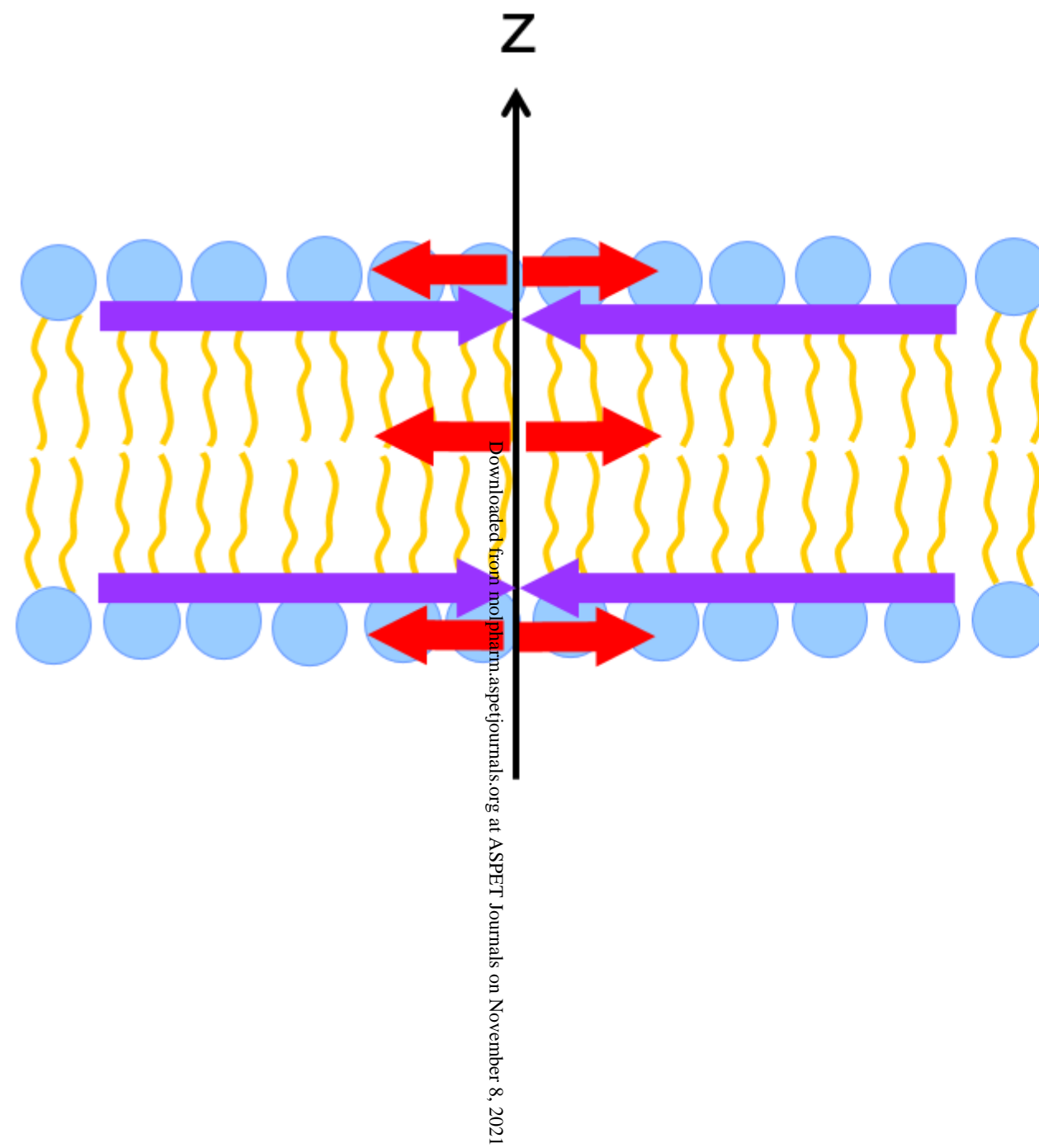


Figure 3



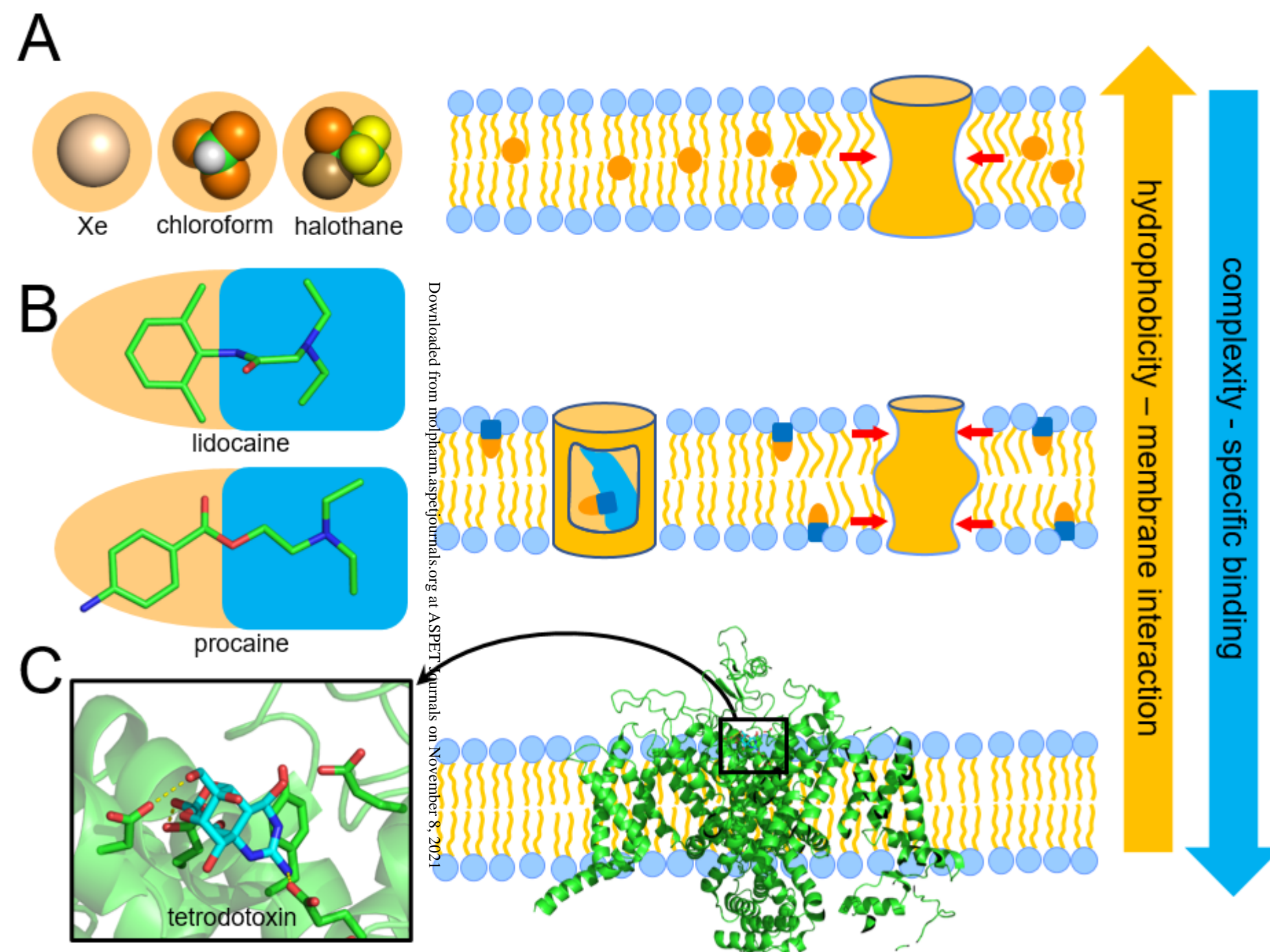


Figure 4

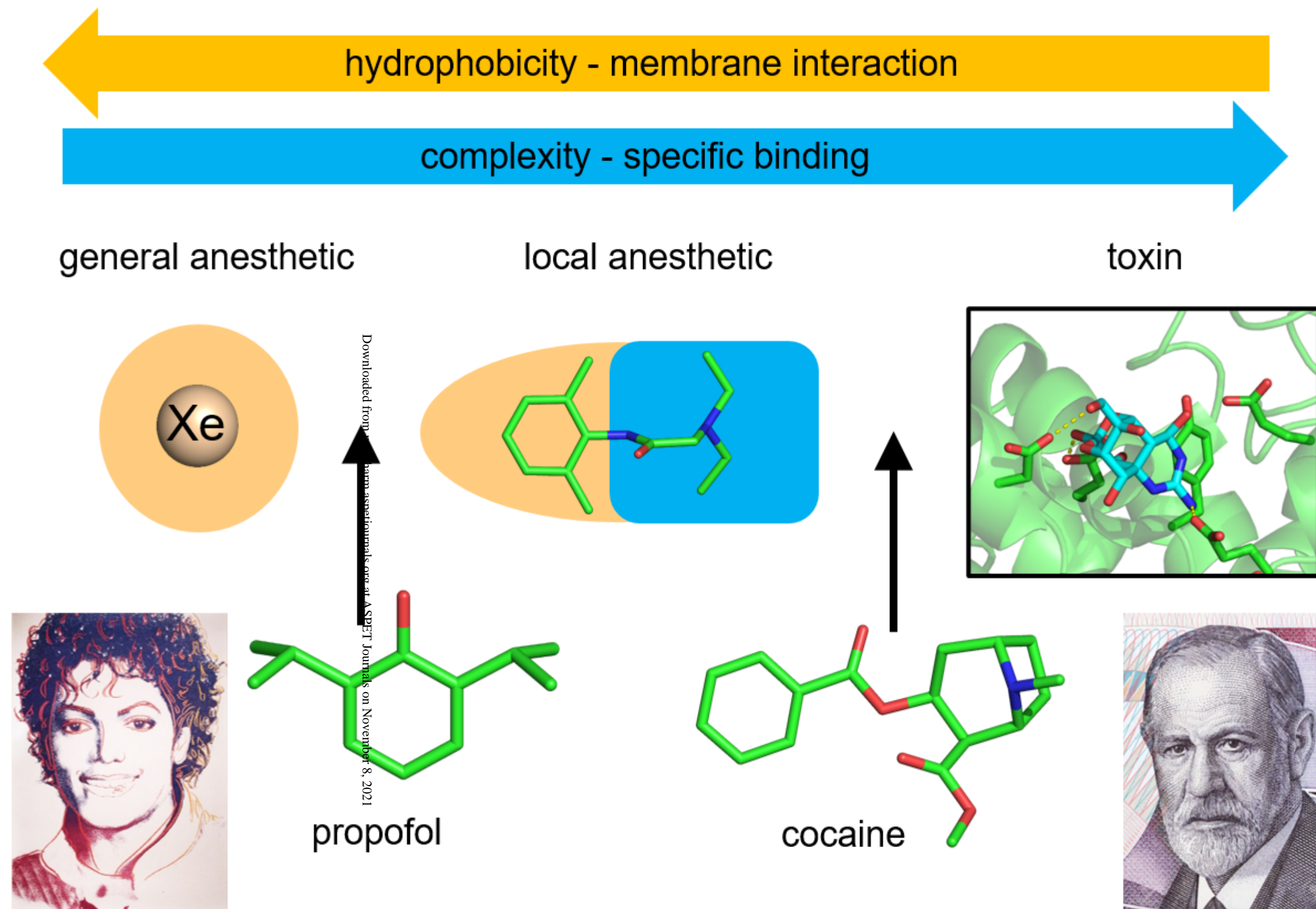


Figure 5