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Computational Studies on the Interactions of Inhalational Anesthetics with Proteins

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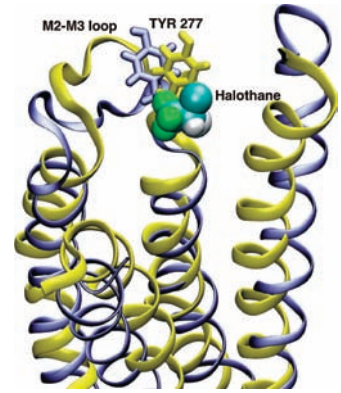

Despite the widespread clinical use of anesthetics since the 19th century, a clear understanding of the mechanism of anesthetic action has yet to emerge. On the basis of early experiments by Meyer, Overton, and subsequent researchers, the cell's lipid membrane was generally concluded to be the primary site of action of anesthetics. However, later experiments with lipid-free globular proteins, such as luciferase and apoferritin, shifted the focus of anesthetic action to proteins. Recent experimental studies, such as photoaffinity labeling and mutagenesis on membrane proteins, have suggested specific binding sites for anesthetic molecules, further strengthening the proteocentric view of anesthetic mechanism. With the increased availability of high-resolution crystal structures of ion channels and other integral membrane proteins, as well as the availability of powerful computers, the structure–function relationship of anesthetic-protein interactions can now be investigated in atomic detail.

In this Account, we review recent experiments and related computer simulation studies involving interactions of inhalational anesthetics and proteins, with a particular focus on membrane proteins. Globular proteins have long been used as models for understanding the role of protein–anesthetic interactions and are accordingly examined in this Account. Using selected examples of membrane proteins, such as nicotinic acetyl choline receptor (nAChR) and potassium channels, we address the issues of anesthetic binding pockets in proteins, the role of conformation in anesthetic effects, and the modulation of local as well as global dynamics of proteins by inhaled anesthetics. In the case of nicotinic receptors, inhalational anesthetic halothane binds to the hydrophobic cavity close to the M2–M3 loop. This binding modulates the dynamics of the M2–M3 loop, which is implicated in allosterically transmitting the effects to the channel gate, thus altering the function of the protein. In potassium channels, anesthetic molecules preferentially potentiate the open conformation by quenching the motion of the aromatic residues implicated in the gating of the channel. These simulations suggest that low-affinity drugs (such as inhalational anesthetics) modulate the protein function by influencing local as well as global dynamics of proteins.

Because of intrinsic experimental limitations, computational approaches represent an important avenue for exploring the mode of action of anesthetics. Molecular dynamics simulations—a computational technique frequently used in the general study of proteins—offer particular insight in the study of the interaction of inhalational anesthetics with membrane proteins.

Introduction

The molecular mechanism of general anesthetics (GA) has remained elusive despite the use of anesthetics over 150 years. The nonspecific or lipid theory dominated the understanding of anesthesia for more than a century.[1] This theory was based on Meyer and Overton observations[2,3] which predicted that the potency of anesthetic molecules strongly correlates with their relative solubility in nonpolar solvents such as olive oil. Under this theory, anesthetic molecules were assumed to dissolve in the cell membrane, per-

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turb the membrane, and thereby alter the cell's functions. The nonspecific theory of anesthesia was supported by many experimental observations. For instance, when anesthetics were added to lipid bilayers, changes were observed in membrane fluidity[4,5] and membrane thickness,[6] the phase diagram of membranes,[7,8] membrane surface dipole potential,[9,10] the bilayer curvature,[11] or lateral pressure.[12] Experiments using nuclear magnetic resonance (NMR) and nuclear Overhauser effect (NOE) techniques showed that the preferential location of anesthetic molecules is at the membrane—water interface, approximately below the head groups of the lipid molecules.[13−15]

Today, the lipid theory is more or less abandoned by researchers with a few exceptions.[12,16] This is in part because the effects of anesthetic molecules on lipid bilayer are so subtle that a small increase in temperature could reproduce similar effects.[17,18] There also exist a number of compounds called nonimmobilizers which are volatile halogenated alkenes with structures and lipophilicities similar to those of GAs,[13,14,19] while lacking anesthetic properties. Finally, the discovery of the inhibition of bioluminescence activity of the lipid-free enzyme luciferase by GAs, helped in bringing the proteocentric phase of anesthetic mechanism to the forefront.[18,20−22]

Current experimental evidence strongly supports proteins rather than the lipid bilayer as the most likely molecular targets for GAs.[23,24] Even though GAs are known to interact with globular proteins directly,[25−28] ligand-gated ion channels (LGIC) of the central nervous system (CNS) are thought to be likely candidates for direct anesthetic action.[24] The nature and viable mechanism of the specific action of GAs on proteins has been discussed extensively.[24,29] Structural details of proteins complexed with anesthetics are required for an atomistic understanding of volatile anesthetic action. Recent experimental techniques like functional and equilibrium binding assays, ^{19}F-NMR spectroscopy, and direct photoaffinity labeling have helped in identifying the probable location of bound GAs and their effect on local and global properties of protein targets.[23,30] Moreover, the function of many ion channels is modified by GAs at clinically relevant concentrations. These ion channels include γ-aminobutyric acid ($GABA_A$), glycine, nicotinic acetylcholine (nACh), 5-hydroxytryptamine ($5\text{-}HT_3$), *N*-methyl-D-aspartate receptors, and voltage-gated Na^+, K^+, and Ca^{2+} channels[31]

Currently, approaches like photoaffinity labeling,[32] kinetic studies,[33] and site-directed mutagenesis of candidate GA targets[34,35] are applied to identify the location of anesthetic binding sites in ion channels. The experimental difficulties of using X-ray crystallography or NMR spectroscopy to LGIC limits obtaining direct structural information about their GA binding sites.[36] In the light of a paucity of such atomistic information, understanding the mechanism of anesthetic effects is difficult. Structures of many globular protein—anesthetic complexes are currently available.[22,26,27,37−39] These globular proteins play either no, or only a small role in CNS signaling, but they have been used as models to delineate the role of specific amino acids on the binding affinity of inhaled anesthetics. Nevertheless, our understanding of the fundamental nature of anesthetic interactions is still limited. GAs are relatively apolar molecules, limiting their interactions with their targets to the hydrophobic effect and weak polarization forces. Since volatile anesthetics are low-affinity ligands, which interact weakly with protein targets, this has hampered direct observation of binding by experiments. The evidence of specific interactions remains at best indirect.[40]

Thus, due to intrinsic experimental limitations, computations can play a pivotal role in exploring the underlying structural features and mode of action of anesthetics. Molecular dynamics simulations (MDS) occupy a central place in the study of proteins. Nowadays, state-of-the-art software and ever increasing CPU power allows microsecond time scales to be accessible for protein and/or lipid systems with explicit solvent water. Recent reviews have described the use of atomistic MDS to study lipid bilayers[41] and ion channels.[42] In this account, we focus on MDS of inhalational anesthetics (IA), a sub class of GAs, with membrane proteins.

Interaction of Anesthetics with Lipid Bilayers

Computer simulations have been carried out using both clinical and higher concentrations of inhalational anesthetic and nonimmobilizer molecules in lipid bilayers. The lipid bilayer models used to probe the distribution of IAs were fully hydrated membranes consisting of dimyristoyl-phosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and stearoyl-docosahexaenoyl—phosphatidylcholine (SDPC).[43−48] These simulations, in agreement with experiments,[13−15] suggest that the anesthetic molecules at clinical concentrations do not alter the overall structure of the lipid bilayer significantly and that IAs preferentially segregate to the outer part of the lipid membrane, near the head-groups.[43] However, at higher concentrations IAs cause significant modifications to the bilayer structure. The most notable changes observed were in the orientation of the headgroup phosphate—nitrogen dipole,

the area per lipid headgroup, the acyl tail mobility, and the existence/presence of gauche rotamer defects.[44] In contrast, nonimmobilizers were preferentially located in the lipid hydrocarbon region, and even at higher concentrations they did not significantly alter the bilayer structure or its fluidity. These differences in the behavior of anesthetic and nonimmobilizer molecules were attributed to the higher hydrophobicity and lack of net dipole moment of the nonimmobilizer.[45,46]

Anesthetics and Globular Proteins

Many globular proteins have been shown to bind anesthetics, and the relative ease of obtaining 3D structures of them complexed with anesthetics helped in gaining valuable insights into anesthetic binding sites. Even though these proteins are unlikely to contribute to anything like anesthesia, valuable information has been obtained about the nature of the cavities where inhalational anesthetics bind, the role of specific amino acids in the binding of anesthetics, and the effects of anesthetics on local and global protein dynamics. The information gathered from the studies on these soluble proteins, described below, suggests that anesthetics preferentially bind in pre-existing cavities, which are of an amphipathic nature.[49]

In luciferase, anesthetics inhibit enzymatic activity.[20] It was suggested that this inhibition was caused by anesthetics competing with the endogenous ligands specific to these binding sites.[18] Computational studies were undertaken by Tang and co-workers using the homodimeric enzyme Δ^5-3-ketosteroid isomerase (KSI),[50] and firefly luciferase[50,51] to explore this proposition. In the first set of computations, MDS coupled with docking studies were carried out on a hydrated KSI in the presence and absence of halothane in order to probe the anesthetic−protein interactions and the anesthetic effects on the activity of KSI.[50] Experimentally, halothane reduces KSI enzymatic activity and perturbs residues in the β-sheet domain of the protein.[52] This finding suggests that anesthetic molecules may affect the enzyme activity by perturbing the quaternary structure of the protein, with similar implications for multidomain receptors in the CNS.

Soluble synthetic α-helix bundles, which are scaled-down representatives of the transmembrane portion of LGICs, have been extensively used experimentally.[53−55] Two 62-residue di-α-helical peptides, in native[56] and mutated[57] forms, in gas-phase and solution were studied using MDS. Simulations were performed in the presence and absence of halothane. The peptide bundle was shown experimentally[58] to have a high affinity for the binding of halothane. Analysis of two MD trajectories revealed that halothane was confined to the hydrophobic core of the peptide bundle. Consistent with experimental observations[58,59] halothane undergoes interactions with Trp residues, which produce pronounced fluorescence quenching. Experimentally, it was observed that the point mutation L38M, increased the binding affinity of halothane by a factor of 3.5 in $(A(\alpha_2)\text{-}L38M)_2$.[59] MDS of the mutated form predicted that this modification was not directly involved in halothane binding.[57] Thus the effect of this mutation on the increased binding affinity of halothane is most likely indirect. A high-resolution NMR structure of the mutant in the presence of halothane confirmed the computational predictions.[55] This signifies the role played by computational approaches in understanding and predicting the atomistic details of mechanisms underlying anesthetic interactions with proteins.

Design and synthesis of a new halothane-binding amphiphilic peptide, with only a single cavity, and an otherwise identical control peptide with no such cavity has been recently carried out by Blasie et al.[60] X-ray reflectivity to monolayers of these peptides was employed to probe the distribution of halothane along the length of the core of these 4-helix bundles as a function of the halothane concentration. In a recent study,[61] spectroscopic methods, coupled with all-atom MDS were carried out to probe the interaction of IA halothane with a model membrane protein. Results of these experiments established the suitability of these model systems and the techniques to work on the mechanism of general anesthesia.

In another recent study,[62] a fluorescent general anesthetic, 1-aminoanthracene (1-AMA), was identified using horse spleen apoferritin (HSAF) via a combination of experimental and modeling work. HSFA is a water-soluble protein that exhibits striking architectural similarities to the transmembrane (TM) region of the superfamily of ligand-gated channels such as the $GABA_A$ receptor. Displacement of 1-AMA from HSAF by other anesthetics attenuates its fluorescence signal and allows determination of kinetic parameters for binding. This provides a unique assay for compound screening and anesthetic discovery.

Membrane Proteins and Anesthetics

Prototypes. Gramicidin A (gA) has been used as a model channel to understand the effects of general anesthetics on protein structure and dynamics both experimentally[63−65] and computationally.[66] Experiments and simulations have shown that low affinity anesthetic molecules like halothane or 1-chloro-1,2,2-trifluorocyclobutane do not affect the secondary structure of gA but have profound effect on the protein

dynamics. Anesthetic molecules alter the dynamics of the channel by significantly modulating the dynamics of tryptophan residues, modifying the protein—lipid interactions, crucial for the functioning of the channel. A recent NMR study[67] on a model membrane protein, Mistic, also suggested that IAs affected the protein dynamics on the microsecond—millisecond time scale while not altering the secondary structure.

K^+ Channels. K^+ channels are perhaps the most extensively studied family of ion channels and there are various examples in which their gating has been shown to be modified by IAs at clinically relevant concentrations.[24] Thus, it has been speculated that they may be pharmacologically important targets of anesthetics.[68] The possible effect of IAs binding to the allosteric sites of ion channels is to modify their gating mechanism, by presumably altering the equilibrium between the open and closed states of the channel. This results in an increase of the channel open probability. Special amino acids that are essential for anesthetic action have been identified, which provides an additional tool for computational investigation of the importance of the target in the effects of anesthetics.[69]

Potassium ion channels can be classified as Kv channels, which are activated by a change in transmembrane voltage, Kir or inward rectifier channels, which preferentially conduct ions into the cell, although this is opposite to the usual physiological direction of potassium flow, and the two pore domain K^+ channels (K2P) so named because their primary sequences contain two pore-forming segments. At present, there are examples of resolved structures for most of the categories in which K^+ channels are classified, which provides a good starting point for computational studies of the interactions of K^+ channels and inhaled anesthetics. All K^+ channels share the same core topology and structure. The channel-forming core is composed of two TM helices separated by a re-entrant loop made of a short pore helix plus a more extended region of polypeptide that forms the selectivity filter. K^+ channels differ in the presence or absence of additional TM helices and additional nonmembrane domains and subunits that control their gating.

Volatile anesthetics at surgical concentrations have been shown to activate various K2P channels such as TASK or TREK-1. The tandem-pore-domain weakly inward rectifying K^+ channel (TWIK) commonly known as TRESK, linked to pain sensation, is sensitive to volatile anesthetics and is thought to play an important role in the mechanism mediating general anesthesia.[70] Stimulatory effects of sevoflurane and enantiomeric isoflurane on human TASK-1 have been observed at clinically relevant concentrations.[71] Some tandem-pore-domain K^+ channels have been shown to be halothane inhibited and are known as THIK.[72,73] The interactions with these K-channels are thought to be largely hydrophobic, although the investigators speculate that some anesthetics may hydrogen-bond to particular grooves of these proteins replacing bound water molecules.[74]

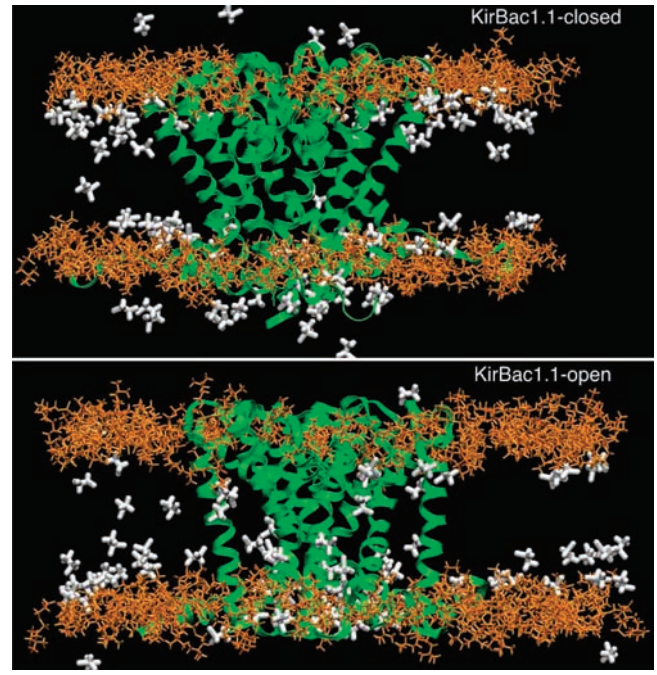

FIGURE 1. Snapshots of the closed and open conformations of KirBac 1.1 channel in the presence of halothane molecules and embedded in a lipid bilayer. The channel is represented in green ribbons and the lipid head groups in orange. The halothane molecules are shown in white.

It has been suggested that anesthetics may prolong the open-state conformation of channels.[75] The availability of an open and a closed structural model for the KirBac1.1 K^+ channel[76,77] permitted a comparative analysis of the interactions of anesthetics with the same channel in two different conformations using computational methods. These studies revealed that the anesthetic molecules modulate the global dynamics of both conformations. The global dynamical motion of the open channel is quenched in the presence of halothane, and a reduction of the flexibility of the inner loops was observed. Anesthetic molecules preferentially target Phe residues close to the open pore (Figure 1); residues that have been previously suggested to be involved in the gating mechanism of the channel.[78]

In agreement with experimental observations,[79] simulations[80] have shown that GAs tend to act primarily on their ion conducting, open conformation. This open conformation exists for just milliseconds, which makes its experimental examina-

tion arduous. Thus computation has played, and will continue to play, a key role in illuminating the atomistic-level understanding of processes linked to general anesthesia.

Nicotinic Acetylcholine Receptors. The nicotinic acetylcholine receptor (nAChR) is a cation selective ion channel, activated by the neurotransmitter acetylcholine (ACh). The atomic structure of the closed form of nACh receptor at 4 Å resolution was solved by electron microscopy.[81,82] It belongs to the superfamily of "cys-loop" LGIC. Other members of this superfamily of LGIC include $GABA_A$, glycine, and serotonin (5-HT3) receptors. Each has a pentameric arrangement of different subunits about a central, ion-conducting axis. Each of the subunits in turn consists of a large N-terminal extracellular domain and four helical TM regions (M1-M4). The amino acid residues in the M2 subunit line the lumen of the channel and residues in M3 and M4 interact with the bilayer. In general, anesthetics potentiate the effect of agonist on $GABA_A$ and glycine receptors and inhibit the nACh receptors.[32] NMR[83,84] and photaffinity labeling experiments[32,85] have shown sensitivity, direct interaction, and multiple binding sites of anesthetic molecules with the transmembrane domain of nAChRs.

MDS were carried out on the membrane bound peptide bundles of α- and δ-subunits in an aqueous-membrane environment[86] to investigate the potential binding of inhalational anesthetic halothane and compare to experiments in order to corroborate the photolabeled sites.[32] Halothane molecules were initially placed in the aqueous phase, and they spontaneously partitioned into the bilayer over a 5 ns time period. The free energy barrier for partitioning halothane molecules into the bilayer was found to be small $\sim$1 kcal/mol, which explains the spontaneous process. Halothane molecules affected the lipid−protein contacts and attenuated local-chain dynamics as was seen in experiments.[87,88] A single halothane molecule was found to enter the α-subunit and it occupied a position consistent to a photolabeled[32] tyrosine residue near the loop connecting M2 and M3 helices. This "bound" halothane molecule decreased the mobility of the tyrosine residue and the flexibility of the loop and significantly affected the correlated motions between helices in the subunit. This affinity of halothane molecules for aromatic residues and their ability to quench the motion of aromatic residues is consistent with many of the observations from simulations described in this account. Earlier it was proposed that the loop connecting M2 and M3 helices plays a key role in the gating mechanism by allosterically affecting the channel gate.[89] A recent NMR study on a Cys-loop prototype 4-α-helix bundle, Mistic,[67] also suggested that one of the primary action sites for inhalational anesthetics is the loop connecting α-helices, consistent with the computational results described previously. The M2−M3 loop was also shown to play a critical role in the correlation between anesthetic binding and neuronal nACh channel's inhibition.[90] Therefore, the observed alteration of dynamics of this loop by bound anesthetic molecules could play a major role in channel inhibition. This modification of protein dynamics by the anesthetics is consistent with the general hypothesis proposed by Tang and co-workers.[91]

Future Outlook. Hundreds of substances have been tested and found to possess anesthetic activity although very few of these have been introduced into clinical practice. At present, the intravenous agent propofol and the inhalational agent sevoflurane seem the preferred anesthetics for induction and for anesthesia maintenance, respectively. Nevertheless, a comprehensive understanding of the mechanism and site of action of anesthetics is still lacking, and the question of how anesthetics act cannot be answered today. The lack of tools to allow the study of these systems experimentally and the complexity of the CNS are two of the primary reasons for this situation.

The present account describes efforts toward understanding modes of action of anesthetics using computational simulations. Though some of the systems described in this account may not play a direct role in anesthesia, general principles have been derived from such models, which have provided the foundation and support the feasibility of future computational studies. One of the key issues is to separate the effects of changes in surrounding lipids due to anesthetics from their direct action on protein function. Development of reliable force fields for various anesthetic molecules to understand their partitioning and interaction with lipid−protein systems is crucial. Currently, working models assume that there are a number of different anesthetic sites in proteins, and the relative affinities of these sites for the anesthetic molecules varies between protein families and protein conformations. Therefore, structural data are required to prove the existence and to define the location of these sites. With the availability of an increasing number of new high-resolution crystal structures, relentless increase in the size and performance of multiprocessor computers, coupled with new algorithms and methods, and on-going development of reliable force fields, computer simulations will keep playing a significant role both in elucidating and describing protein−anesthetic interactions at an atomistic level, and potentially they should aid in the design of new anesthetics agents.[62]

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BIOGRAPHICAL INFORMATION

Satyavani Vemparala received a M.Sc. and Ph.D. in Physics from University of Hyderabad, India, and Louisiana State University, U.S.A., respectively. She worked with Priya Vashishta on simulating self-assembled monolayers during her Ph.D. She was a postdoctoral fellow with Michael L. Klein at the University of Pennsylvania working on variety of topics including antimicrobial polymers, ion channels, effects of small drug molecules on lipid bilayers. Since 2006 she is a faculty fellow at The Institute of Mathematical Sciences, Chennai, India.

Carmen Domene received a B.Sc. degree in Chemistry from the University of Seville, Spain. She received a Ph.D. in Chemistry from the University of Exeter, U.K., where she worked with Patrick Fowler and Paul Madden on modeling many-body effects in interionic interactions. She then moved to the University of Oxford, U.K. as a postdoctoral fellow at the Laboratory of Molecular Biophysics, working on computer simulations of ion channels. Since 2003, she holds a Royal Society University Research fellowship at the Physical and Theoretical Chemistry Laboratory of the University of Oxford. She has been a regular visiting fellow in the Klein group since 2004.

Michael L. Klein received a B.Sc. and Ph.D. in Chemistry from the University of Bristol, U.K.. He was a postdoctoral fellow in Italy, U.K., and U.S.A. before joining the Chemistry Division of the NRCC in Ottawa, Canada, where he rose through the ranks from Associate to Principal Research Officer. In 1987, he returned to the United States as Professor of Chemistry at the University of Pennsylvania. Since 1993, he has been Hepburn Professor of Physical Science and Director of the Laboratory for Research on the Structure of Matter. His current research involves probing the structure and dynamics of molecular systems using computer simulation techniques; systems of interest include aqueous solutions, model membranes, and membrane-bound proteins.

FOOTNOTES

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REFERENCES

1 Campagna, J. A.; Miller, K. W.; Forman, S. A. Mechanisms of actions of inhaled anesthetics. *New Engl. J. Med.* **2003**, *348*, 2110–2124.

2 Meyer, K. H. Contribution to the theory of narcosis. *Trans. Faraday Soc.* **1937**, *33*, 1062–1068.

3 Overton, E. *Studien uber die Narkose, Zugleich ein Beitrag zur Allegemeiner Pharmakologie*; Gustav Fischer: Jena, Switzerland, 1901.

4 Firestone, L. L.; Alifimoff, J. K.; Miller, K. W. Does general anesthetic-induced desensitization of the *torpedo* acetylcholine receptor correlate with lipid disordering. *Mol. Pharmacol.* **1994**, *46*, 508–515.

5 Ueda, I.; Hirakawa, M.; Arakawa, K.; Kamaya, H. Do anesthetics fluidize membranes. *Physiology* **1986**, *28*, 292–299.

6 Haydon, D. A.; Hendry, B. M.; Levinson, S. R.; Requena, J. Anaesthesia by the *n*-alkanes. A comparitive study of nerve impulse blockage and the properties of black lipid bilayer membranes. *Biochim. Biophys. Acta* **1977**, *470*, 17–34.

7 Galla, H. J.; Trudell, J. R. Asymmetric antagonistic effects of an inhalational anestehtic and high pressure on the phase transition temperature of dipalmitoyl phosphatidic acid bilayers. *Biochim. Biophys. Acta* **1980**, *599*, 336–340.

8 Vanderkooi, J. M.; Landesberg, R.; Selick, H.; McDonald, G. G. Interaction of general anesthetics with phospholipid vesicles and biological membranes. *Biochim. Biophys. Acta* **1977**, *464*, 1–18.

9 Reyes, J.; Latorre, R. Effect of the anesthetics benzyl alcohol and chloroform on bilayers made from monolayers. *Biophys. J.* **1979**, *28*, 259–279.

10 Qin, Z.; Szabo, G.; Cafiso, D. S. Anesthetics reduce the magnitude of the membrane dipole potential. Measurements in lipid vesicles using voltage-sensitive spin probes. *Biochemistry* **1995**, *34*, 5536–5543.

11 Gruner, S. M.; Shyamsunder, E. Is the mechanism of general anesthesia related to lipid membrane spontaneous curvature. *Ann. New York Acad. Sci.* **1991**, *625*, 685–697.

12 Cantor, R. S. The lateral pressure profile in membranes: A physical mechanism of general anesthesia. *Biochemistry* **1997**, *36*, 2339–2344.

13 North, C.; Cafiso, D. Contrasting membrane localization and behavior of halogenated cyclobutanes that follow or violate the Meyer—Overton hypothesis of general anesthetic potency. *Biophys. J.* **1997**, *72*, 1754–1761.

14 Tang, P.; Yan, B.; Xu, Y. Different distribution of fluorinated anesthetics and non-anesthetics in model membranes a 19F NMR study. *Biophys. J.* **1997**, *72*, 1676–1682.

15 Baber, J.; Ellena, J. F.; Cafiso, D. Distribution of general anesthetics in phospholopid bilayers determined using ^{2}H NMR and ^{1}H—^{1}H NOE spectroscopy. *Biochemistry* **1995**, *34*, 6533–6539.

16 Cantor, R. S. Lipid composition and the lateral pressure profile in bilayers. *Biophys. J.* **1999**, *76*, 2625–2639.

17 Franks, N. P.; Lieb, W. R. Molecular mechanisms of general anaesthesia. *Nature* **1982**, *300*, 487–493.

18 Franks, N. P.; Lieb, W. R. Do general anaesthetics act by competitive binding to specific receptors. *Nature* **1984**, *310*, 599–601.

19 Koblin, D. D.; Chortkoff, B. S.; Laster, M. J.; Eger, E. I.; Halsey, M.; Ionescu, P. Polyhalogenated and perflourinated compounds that disobey the Meyer—Overton hypothesis. *Anesth. Analg.* **1994**, *79*, 1043–1048.

20 Ueda, I. Effects of diethyl ether and halothane on firefly luciferin bioluminiscence. *Anesthesiology* **1965**, *26*, 603–606.

21 Ueda, I.; Suzuki, A. Irreversible phase transition of firefly Luciferase: contrasting effects of volatile anesthetics and myristic acid. *Biochim. Biophys. Acta* **1998**, *1380*, 313–319.

22 Franks, N. P.; Jenkins, A.; Conti, E.; Lieb, W. R.; Brick, P. Structural basis for the inhibition of firefly luciferase by a general anesthetic. *Biophys. J.* **1998**, *75*, 2205–2211.

23 Eckenhoff, R. G.; Johansson, J. S. Molecular interactions between inhaled anesthetics and proteins. *Pharmacol. Rev.* **1997**, *49*, 343–367.

24 Franks, N. P. General anaesthesia: From molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.* **2008**, *9*, 370–386.

25 Dubois, B. W.; Cherian, S. F.; Evers, A. S. Volatile anesthetics compete for common binding sites on bovine serum albumin: a 19F-NMR study. *Proc. Natl. Acad. Sci., U.S.A.* **1993**, *90*, 6478–6482.

26 Eckenhoff, R. G.; Petersen, C. E.; Ha, C. E.; Bhagavan, N. V. Inhaled anesthetic binding sites in human serum albumin. *J. Biol. Chem.* **2000**, 275.

27 Bhattacharya, A. A.; Curry, S.; Franks, N. P. Binding of the General Anesthetics Propofol and Halothane to Human Serum Albumin. *J. Biol. Chem.* **2000**, *275*, 38731–38738.

28 Eckenhoff, M. F. Promiscuous ligands and attractive cavities: how do the inhaled anesthetics work. *Mol. Interventions* **2001**, *1*, 258–268.

29 Alkire, M. T.; Hudetz, A. G.; Tononi, G. Consciousness and Anesthesia. *Science* **2008**, *322*, 876–880.

30 Evers, A. S.; Crowder, C. M. In *Clinical Anesthesia*; Barash, P. G., Cullen, B. F., Stoelting, R. K., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; pp 111—132.

31 Franks, N. P. Molecular targets underlying general anesthesia. *Br. J. Pharmacol.* **2006**, *147*, S72–S81.

32 Chiara, D. C.; Dangott, L. J.; Eckenhoff, R. G.; Cohen, J. B. Identification of nicotinic acetylcholine receptor amino acids photolabeled by the volatile anesthetic halothane. *Biochemistry* **2003**, *42*, 13457–13467.

33 Dilger, J. P. The effects of general anesthetics on ligand-gates ion channels. *Br. J. Anaesth.* **2002**, *89*, 41–51.

34 Mihic, S. J.; Ye, Q.; Wick, M. J.; Koltchine, V. V.; Krasowski, M. A.; Finn, S. E.; Mascia, M. P.; Valenzuela, C. F.; Hanson, K. K.; Greenblatt, E. P.; Harris, R. A.; Harrison, N. L. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. *Nature* **1997**, *389*, 385–389.

35 Trudell, J. R.; Bertaccini, E. Molecular modelling of specific and non-specific anesthetic interactions. *Br. J. Anaesth.* **2002**, *89*, 32–40.

36 Miller, K. W. The nature of sites of general anesthetic action. *Br. J. Anaesth.* **2002**, *89*, 17–31.

37 Tilton, R. F.; Kuntz, I. D.; Petsko, G. A. Cavities in proteins: structure of a metmyoglobin-xenon complex solved to 1.9 Å. *Biochemistry* **1984**, *23*, 2849–2857.

38 Colloc'h, N.; Santos, J. S. O.; Retailleau, P.; Vivarès, D.; Bonneté, F.; d'Estainto, B. L.; Gallois, B.; Brisson, A.; Risso, J.; Lemaire, M.; Prangé, T.; Abraini, J. H. Protein crystallography under xenon and nitrous oxide pressure: Comparison with in vivo pharmacology studies and implications for the mechanism of inhaled anesthetic action. *Biophys. J.* **2007**, *92*, 217–224.

39 Liu, R.; Loll, P. J.; Eckenhoff, R. G. Structural basis for high affinity volatile anesthetic binding in a natural 4-helix bundle protein. *FASEB J.* **2005**, *19*, 567–576.

40 Urban, B. W. The site of anesthetic action. *Handb. Exp. Pharmacol.* **2008**, *182*, 3–29.

41 Edholm, O. In *Computational Modeling of Membrane Bilayers*; Feller, S. E., Ed.; Academic Press: New York, 2008; Vol. 60, pp 91—110.

42 Domene, C. Molecular dynamics simulations of potassium channels. *Cent. Eur. J. Chem.* **2007**, *5*, 635–671.

43 Tu, K. C.; Tarek, M.; Klein, M. L.; Scharf, D. Effects of anesthetics on the structure of a phospholipid bilayer: Molecular dynamics investigation of halothane in the hydrated liquid crystal phase of dipalmitoylphosphatidylcholine. *Biophys. J.* **1998**, *75*, 2123–2134.

44 Koubi, L.; Tarek, M.; Klein, M. L.; Scharf, D. Distribution of halothane in a dipalmitoylphosphatidylcholine bilayer from molecular dynamics calculations. *Biophys. J.* **2000**, *78*, 800–811.

45 Koubi, L.; Saiz, L.; Tarek, M.; Scharf, D.; Klein, M. L. Influence of anesthetic and nonimmobilizer molecules on the physical properties of a polyunsaturated lipid bilayer. *J. Phys. Chem. B* **2003**, *107*, 14500–14508.

46 Koubi, L.; Tarek, M.; Bandyopadhyay, S.; Klein, M. L.; Scharf, D. Membrane structural perturbations caused by anestehtics and nonimmobilizer: a molecular dynamics investigation. *Biophys. J.* **2001**, *81*, 3339–3345.

47 Koubi, L.; Tarek, M.; Bandyopadhyay, S.; Klein, M. L.; Scharf, D. Effects of the nonimmobilizer hexafluroethane on the model membrane dimyristoylphosphatisylcholine. *Anesthesiology* **2002**, *97*, 848–855.

48 Saiz, L.; Klein, M. L. Computer simulation studies of model biological membranes. *Acc. Chem. Res.* **2002**, *35*, 482–489.

49 Bertaccini, E.; Trudell, J. R.; Franks, N. P. The common chemical motifs within anesthetic binding sites. *Anesth. Analg.* **2007**, *104*, 318–324.

50 Yonkunas, M. J.; Xu, Y.; Tang, P. Anesthetic interaction with ketosteroid isomerase: insights from molecular dynamics simulations. *Biophys. J.* **2005**, *89*, 2350–2356.

51 Szarecka, A.; Xu, Y.; Tang, P. Dynamics of firefly Luciferase inhibition by general anesthetics: gaussian and anisotropic network analysis. *Biophys. J.* **2007**, *93*, 1895–1905.

52 Yonkunas, M. J.; Ma, D.; Liu, Z.; Li, L.; Xu, Y.; Tang, P. Allosteric low-affinity drug action on ketosteroid isomerase. *Biophys. J.* **2004**, *86*, 514a.

53 Pidikiti, R.; Shamim, M.; Mallela, K. M. G.; Reddy, K. S.; Johansson, J. S. Expression and characterization of a four-alpha-helix bundle protein that binds the volatile general anesthetic halothane. *Biomacromolecules* **2005**, *6*, 1516–1523.

54 Ma, D.; Brandon, N. R.; Cui, T.; Bondarenko, V.; Canlas, C.; Johansson, J. S.; Tang, P.; Xu, Y. Four-{alpha}-Helix Bundle with Designed Anesthetic Binding Pockets. Part I: Structural and Dynamical Analyses. *Biophys. J.* **2008**, *94*, 4454–4463.

55 Cui, T.; Bondarenko, V.; Ma, D.; Canlas, C.; Brandon, N. R.; Johansson, J. S.; Tang, P.; Xu, Y. Four-{alpha}-Helix Bundle with Designed Anesthetic Binding Pockets. Part II: Structural and Dynamical Analyses. *Biophys. J.* **2008**, *94*, 4464–4472.

56 Davies, L. A.; Klein, M. L.; Scharf, D. Molecular dynamics simulation of a synthetic four-alpha-helix bundle that binds the anesthetic halothane. *Febs Lett.* **1999**, *455*, 332–338.

57 Davies, L. A.; Zhong, Q. F.; Klein, M. L.; Scharf, D. Molecular dynamics simulation of four-alpha-helix bundles that bind the anesthetic halothane. *Febs Lett.* **2000**, *478*, 61–66.

58 Johansson, J. S.; Gibney, B. R.; Rabanal, F.; Reddy, K. S.; Dutton, P. L. A designed cavity in the hydrophobic core of a four-alpha-helix bundle improves volatile anesthetic binding affinity. *Biochemisry* **1998**, *37*, 1421–1429.

59 Johansson, J. S.; Scharf, D.; Davies, L. A.; Reddy, K. S.; Eckenhoff, R. G. A Designed Four-alpha -Helix Bundle That Binds the Volatile General Anesthetic Halothane with High Affinity. *Biophys. J.* **2000**, *78*, 982–993.

60 Churbanova, I. Y.; Tronin, A.; Strazalka, J.; Gog, T.; Kuzmenko, I.; Johansson, J. S.; Blasie, J. K. Monolayers of a model anesthetic-binding membrane protein. Formation, characterization and halothane-binding affinity. *Biophys. J.* **2006**, *90*, 3255–3266.

61 Zou, H.; Liu, J.; Blasie, J. K. Mechanism of interaction between the general anesthetic halothane and a model ion channel protein III: Molecular Dynamics Simulation Incorporating a Cyanophenylalanine Spectroscopic Probe. *Biophys. J.* **2009**, *96*, 4188–4199.

62 Butts, C. A.; Xi, J.; Brannigan, G.; Klein, M. L.; Eckenhoff, R. G.; Dmochowski, I. J. Identification of a fluorescent general anesthetic, 1-aminoanthracen. *Proc. Natl. Acad. Sci., U.S.A.* **2009**, *106*, 6501–6506.

63 Tang, P.; Mandal, P. K.; Zegarra, M. Effects of volatile anesthetic on channel structure of gramicidin A. *Biophys. J.* **2002**, *83*, 1413–1420.

64 Tang, P.; Eckenhoff, R. G.; Xu, Y. General anesthetic binding to gramicidin A: The structural requirements. *Biophys. J.* **2000**, *78*, 1804–1809.

65 Tang, P.; Simplaceanu, V.; Xu, Y. Structural consequences of anesthetic and nonimmobilizer interaction with gramicidin A channels. *Biophys. J.* **1999**, *76*, 2346–2350.

66 Liu, Z. W.; Xu, Y.; Tang, P. Molecular dynamics simulations of C_2F_6 effects on gramicidin A: Implications of the mechanisms of general anesthesia. *Biophys. J.* **2005**, *88*, 3784–3791.

67 Canlas, C. G.; Cui, T. X.; Li, L.; Xu, Y.; Tang, P. Anesthetic modulation of protein dynamics: Insight from an NMR study. *J. Phys. Chem. B* **2008**, *112*, 14312–14318.

68 Patel, A. J.; Honore, E.; Lesage, F.; Fink, M.; Romey, G.; Lazdunski, M. Inhalational anesthetics activate two-pore-domain background K+ channels. *Nat. Neurosci.* **1999**, *2*, 422–426.

69 Andres-Enguix, I.; Caley, A.; Yustos, R.; Schumacher, M. A.; Spanu, P. D.; Dickinson, R.; Maze, M.; Franks, N. P. Determinants of the anesthetic sensitivity of two-pore domain acid-sensitive potassium channels—Molecular cloning of an anesthetic-activated potassium channel from Lymnaea stagnalis. *J. Biol. Chem.* **2007**, *282*, 20977–20990.

70 Huang, D. Y.; Yu, B. W.; Fan, Q. W. Roles of TRESK, a novel two-pore domain K+ channel, in pain pathway and general anesthesia. *Neurosci. Bull.* **2008**, *24*, 166–172.

71 Putzke, C.; Hanley, P. J.; Schlichthorl, G.; Preisig-Muller, R.; Rinne, S.; Anetseder, M.; Eckenhoff, R. G.; Berkowitz, C.; Vassiliou, T.; Wulf, H.; Eberhart, L. Differential effects of volatile and intravenous anesthetics on the activity of human TASK-1. *Am. J. Physiol.—Cell Physiol.* **2007**, *293*, C1319–C1326.

72 Yost, C. S. Tandem pore domain K channels: an Important site of volatile anesthetic action. *Curr. Drug Targets* **2000**, *1*, 207–217.

73 Gray, A. T.; Zhao, B. B.; Kindler, C. H.; Winegar, B. D.; Mazurek, M. J.; Xu, J.; Chavez, R. A.; Forsayeth, J. R.; Yost, C. S. Volatile anesthetics activate the human tandem pore domain baseline K+ channel KCNK5. *Anesthesiology* **2000**, *92*, 1722–1730.

74 Harrison, N.; Nau, C. Sensitization of nociceptive ion channels by inhaled anesthetics—A pain in the gas? *Mol. Pharmacol.* **2008**, *74*, 1261–1268.

75 Franks, N. P.; Lieb, W. R. Inhibitory synapses anaesthetics set their sites on ion channels. *Nature* **1997**, *389*, 334–335.

76 Domene, C.; Doyle, D. A.; Venien-Bryan, C. Modeling of an ion channel in its open conformation. *Biophys. J.* **2005**, *89*, L01–L03.

77 Kuo, A.; Domene, C.; Johnson, L. N.; Doyle, D. A.; Venien-Bryan, C. Two different conformational states of the KirBac3.1 potassium channel revealed by electron crystallography. *Structure* **2005**, *13*, 1463–1472.

78 Domene, C.; Vemparala, S.; Klein, M. L.; Venien-Bryan, C.; Doyle, D. A. Role of aromatic localization in the gating process of a potassium channel. *Biophys. J.* **2006**, *90*, L01–L03.

79 Forman, S. A.; Miller, K. W.; Yellen, G. A discrete site for general anesthetics on a polysynaptic receptor. *Mol. Pharmacol.* **1995**, *48*, 574–581.

80 Vemparala, S.; Domene, C.; Klein, M. L. Interaction of anesthetics with open and closed conformations of a potassium channel studied via molecular dynamics and normal mode analysis. *Biophys. J.* **2008**, *94*, 4260–4269.

81 Miyazawa, A.; Fujiyoshi, Y.; Unwin, N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **2003**, *423*, 949–955.

82 Unwin, N. Refined structure of the nicotinic acetylcholine receptor at 4 A resolution. *J. Mol. Biol.* **2005**, *346*, 967–989.

83 Xu, Y.; Seto, T.; Tang, P.; Firestone, L. NMR study of volatile anesthetic binding to nicotinic acetylcholine receptors. *Biophys. J.* **2000**, *78*, 746–751.

84 Bondarenko, V.; Yushmanov, V. E.; Xu, Y.; Tang, P. NMR study of general anesthetic interaction with nAChR beta(2) subunit. *Biophys. J.* **2008**, *94*, 1681–1688.

85 Eckenhoff, R. G. An inhalational anesthetic binding domain in the nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2807–2810.

86 Vemparala, S.; Saiz, L.; Eckenhoff, M. F.; Klein, M. L. Partitioning of anesthetics into a lipid bilayer and their interaction with membrane-bound peptide bundles. *Biophys. J.* **2006**, *91*, 2815–2825.

87 Johansson, J. S.; Zou, H.; Tanner, J. W. Bound volatile general anesthetics alter both local protein dynamics and global protein stability. *Anesthesiology* **1999**, *90*, 235–245.

88 Eckenhoff, R. G. Do specific or nonspecific interactions with proteins underlie inhalational anesthetic action. *Mol. Pharmacol.* **1998**, *54*, 610–615.

89 Law, R. J.; Henchman, R. H.; McCammon, J. A. A gating mechanism proposed from a simulation of a human 7 nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6813–6818.

90 Downie, D. L.; Vincente-Agullo, F.; Campos-Caro, A.; Bushell, T. J.; Lieb, W. R.; Franks, N. P. Determinants of the anesthetic sensitivity of neuronal nicotinic acetylcholine receptors. *J. Biol. Chem.* **2002**, *277*, 10367–10373.

91 Tang, P.; Xu, Y. Large-scale molecular dynamics simulations of general anesthetic effects on the ion channel in the fully hydrated membrane: The implication of molecular mechanisms of general anesthesia. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16035–16040.