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A new understanding of inert gas narcosis*

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Anesthetics are extremely important in modern surgery to greatly reduce the patient's pain. The understanding of anesthesia at molecular level is the preliminary step for the application of anesthetics in clinic safely and effectively. Inert gases, with low chemical activity, have been found to cause anesthesia for centuries, but the mechanism is unclear yet. In this review, we first summarize the progress of theories about general anesthesia, especially for inert gas narcosis, and then propose a new hypothesis that the aggregated rather than the dispersed inert gas molecules are the key to trigger the narcosis to explain the steep dose-response relationship of anesthesia.

Keywords: cluster, inert gas, anesthesia, narcotic potency

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1. Introduction

It has long been accepted that the nanoparticles hardly enter the blood circulation system and tissues of living animals, let alone the cells. Thus, most of the previous experiments are *in vitro* or focus on the damage to the lung and gastrointestinal tract, or even directly inject the nanoparticles into the blood vessel of living animals.[1–13] In contrast, the small gas molecules can easily dissolve into the blood in alveolus with our breathing. When the partial pressure of gases increases, the concentration of gas molecules dissolved in blood increases.

According to the effects to animals, gases can be divided into three kinds roughly. The first kind of gases can participate in the metabolism (oxygen) to generate the metabolic products (carbon dioxide, ethanol) or regulate the physiological processes (Nitric oxide).[14] In normal physiological level, these gases are harmless to body. The second kind of gases can react with the components of living body and cause damage to cells or even lead to the death of living creatures (i.e., carbon monoxide, ammonia gas, hydrogen sulfide, benzene, formaldehyde, and so on). The third kind of gases is the biological inert gas. Biological inert gases mean they are all inert biologically, and exert their effects without changing their own chemical structures or the primary chemical structures of biological tissues, including helium, neon, argon, krypton, xenon, hydrogen, nitrous oxide, and so on. Nearly all the biological inert gases at high partial pressure can cause anesthesia.[15] In this paper, we will briefly review the history of anesthesia, especially inert gas narcosis, and the developing of the theories about general anesthesia. We also present our hypothesis that the inert gases may exert their effects as clusters, which can explain some difficulties explained hardly by previous theories.

Nitrogen narcosis has been well known for centuries. In 1835, Junod noted that when breathing compressed air 'the functions of the brain are activated, imagination is lively, thoughts have a peculiar charm and, in some persons, symptoms of intoxication are present.'[16] While diving at depth more than 30–40 m, the divers inhale compressed air and will be involved into nitrogen narcosis, like drinking alcohol.[15] The degree of nitrogen narcosis will increase along the depth increasing and will make the diving at depth more than 40 meter a dangerous behavior.

In 1844, Horace Wells used nitrous oxide in dental surgery to relax patients.[17] This is the first time for the inert gas to be used as anesthetic in history. Until nowadays, the nitrous oxide is still the unique gas anesthetic often used in surgeries. Due to the high concentration (105%) needed by nitrous oxide anesthesia, it usually mixed with other anesthetics when used in surgeries.

Xenon was discovered by Ramsay and Travers in 1898. It is very rare in nature and only constitutes 0.0000087% of the atmosphere.[18] When xenon is account for 71% of inhaled gases, human will lost consciousness.[19] The blood/gas

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partition coefficient of xenon is only 0.115, which means xenon is the least soluble gas that can be directly used for anesthesia.[20] Xenon was shown to have anesthetic properties in mice in 1946,[21] and Stuart Cullen and Erwin Gross anesthetized two patients with it in 1951.[22] Although the price of xenon limits its widespread application in anesthesia, scientists still think it is the anesthetic for the 21st century. Compared to other anesthetics commonly used in anesthesia, xenon is totally inert to our body, which means that it nearly does not cause side effects.[23] Because of its low solubility in blood, the unconsciousness and weakness for xenon anesthesia is very rapid.

The strengths of anesthesia for different kinds of inert gases vary greatly. To determine the narcotic potency of anesthetics quantitatively, Eger *et al.* proposed a technique, called MAC (minimum alveolar concentration).[24] MAC is the partial pressure (concentration) of the vapor in the lungs that is required to prevent movement (motor response) in 50% of subjects in response to surgical (pain) stimulus. MAC was found to be fairly constant for any subject. The narcotic potency is defined as the reciprocal of MAC (1/MAC). The relative narcotic potency is the relative value when we set the narcotic potency of nitrogen as 1.

Along with the application of anesthetics in clinic, the exploration of the mechanism of anesthesia never stops. In history, two main theories were proposed to explain the mechanism of inert gas anesthesia, the lipid hypotheses and the protein hypotheses.

2. Lipid hypotheses

Early in 1847, von Bibra and Harless proposed the first hypothesis of anesthesia.[25] They thought the anesthetics dissolved into the fat fraction of brain cells and caused the anesthesia. From 1899 to 1901, Mayer and Overton reported their experimental findings in succession.[26–29] They compared the concentrations required to induce anesthesia of several kinds of anesthetics and their olive oil/water partition coefficient and found that for kinds of anesthetics it is a nearly inversely proportional relationship between them. Figure 1 shows the linear correlation between the narcotic potency and the oil/water partition coefficient for inert gases. Helium and Neon usually are thought as nonanesthetics.[15]

In the following 80 years, many scientists proposed their theories according to the Mayer–Overton correlation. They thought the anesthetics might (i) change bilayer dimensions (volume or thickness),[30,31] (ii) increase bilayer fluidity,[32] (iii) induce phase transitions in bilayer lipids,[33,34] (iv) alter bilayer permeability.[35] However, up to now, the lipid hypotheses have been discarded. The lipid hypotheses encountered troubles to explain the findings in experiments. There are some objections to Lipid hypotheses. The direct objection is that detectable changes in bilayer structure cannot be observed at clinically relevant concentrations in experiments.[36] Lipid hypotheses also predict that the MAC decreases with the rise of temperature. However, in fact the opposite is true.[37,38]

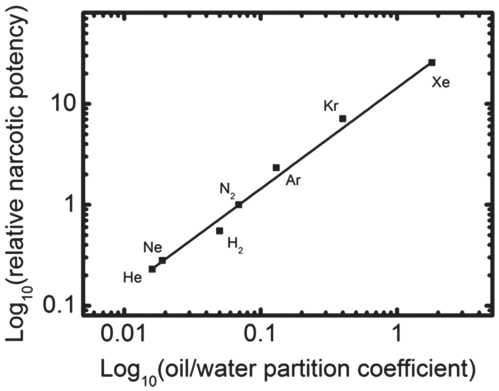


Fig. 1. The linear correlation between the narcotic potency and the oil/water partition coefficient for some inert gases. Data from Ref. [15].

3. Protein hypotheses

In 1984, Franks and Lieb found that the activity of a pure solute protein (firefly luciferase) can be inhibited by 50% at surgical level of halothane.[39] This finding inspired them that anesthesia might be caused by the direct binding of anesthetic molecules to proteins. After that, scientists turn their attention to the protein hypothesis of anesthesia. The most important question became that which proteins are the target proteins for anesthetics. Scientist have found that most general anesthetics can enhance the activity of inhibitory $GAGA_A$ (g-aminobutyric acid type-A) and glycine receptor.[40,41] But for different anesthetics, the target proteins might be also different. In 1998, Franks and his coworkers found in experiment that 80% xenon reduced NMDA-activated (N-methyl-D-aspartate) currents by about 60%, but the effects of Xenon to $GABA_A$ receptors are negligible.[42]

4. Our hypothesis

However, the protein hypothesis is not perfect. An unexplained phenomenon for protein hypotheses is the steep dose-response relationship (Fig. 2(b)).[43] The slope of the dose-response curves of inhalation anesthetics is extraordinarily steep around the MAC of inhalation anesthetics (Fig. 2(b)). Anesthesia usually happened under high partial pressure and the steep dose-response relationship of concentration stimulates us to consider what really happened at the critical anesthetic concentration. From the aspect of response curves, it is very like a phase transition. We guess that anesthesia might be caused by the nucleation of anesthetics, especially for inert gases, which have low affinity to protein.

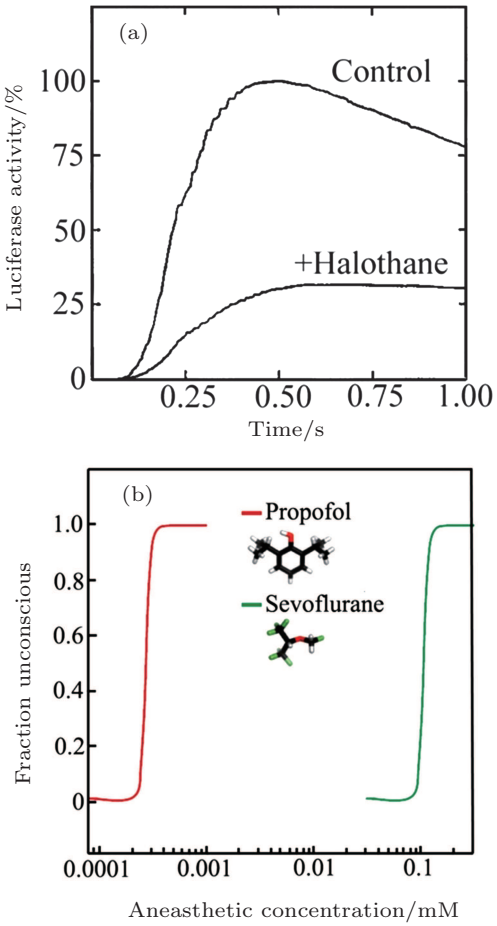


Fig. 2. (a) Inhibition of luciferase activity at surgical levels of halothane. The curves show luciferase activity as a function of time. (b) The concentration–response curves for anaesthetic-induced loss of consciousness are extremely steep. Panel (a) is reprinted by permission from Nature Publishing Group Ltd: [Nature] (Ref. [39]) copyright (1984). Panel (b) is reprinted by permission from Nature Publishing Group Ltd: [Nature Reviews Neuroscience] (Ref. [42]) copyright (2008).

Indeed, the aggregation of gas molecules in nanoscale has been observed experiments in recently years. In 2000, Lou *et al.* first reported the observation of nanobubble at the interface solid and water by atomic force microscopy.[44] Later, many more evidences from experiments have demonstrated the existing of nanobubble and some theories were proposed to understand the stability of nanobubbles.[45,46,57] Since 2006, Fang and his coworkers have proposed that the nanobubble may have a high density inside and the high inert density can enhance the life time of nanobubble.[58–60]

In 2013, we compared the affinities of nitrogen cluster and dispersed nitrogen molecules to SH3 domain (Fig. 3).[61] The Src-homology 3 (SH3) domains are small protein-interaction modules of about 60 residues found in a large number of eukaryotic cytoskeletal and signaling proteins and generally bind proline-rich sequences containing the characteristic PxxP motif.[62–65] The nitrogen molecules can accumulate at the hydrophobic area of protein surface. We used two systems with different concentrations of nitrogen molecules. The first system has a high concentration of N_2 in solution, labeled NS. The nitrogen molecules gathered quickly and adsorb on the SH3. As a control, DS has low concentration of N_2 in solution. The nitrogen molecules remain dispersed in whole simulation. Aggregated N_2 molecules were strongly bound by the active sites of the SH3 domain, which could impair the activity of the protein. In contrast, dispersed N_2 molecules did not specifically interact with the SH3 domain. The binding sites are totally covered the four key binding sites (8(PHE), 36(TRP), 50(PRO), and 53(TYR)) of the SH3 domain.[65]

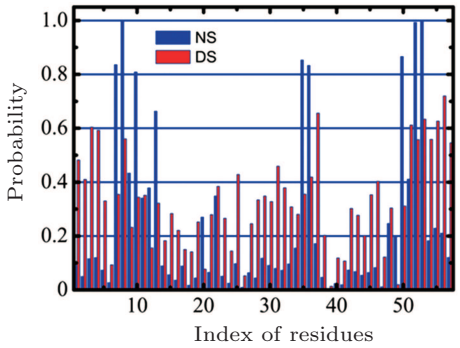


Fig. 3. A comparison of the nitrogen cluster system and the dispersed nitrogen molecules system. The *X*-axis represents the index of residues in the SH3 domain while the *Y*-axis represents the probability of adsorption that residue adsorbed nitrogen molecules. Reprinted by permission from Nature Publishing Group Ltd: [Scientific Reports] (Ref. [43]) copyright (2013).

In reality, when animals inhaled anesthetic with a concentration higher than MAC, they would lost consciousness and sensibility to pain. When the inhaled concentration of anesthetic decreased to lower that MAC, the animals can awake quickly. This fact suggests the process of anesthesia should be reversible, which means that the anesthetics cannot produce irreversible change to animal's body. To show the influences of nitrogen cluster binding to the SH3 domain more clearly, we found a sample in which the cluster of nitrogen departed from the SH3 domain at last. In this sample, the nitrogen molecules aggregated quickly in 10 ns at the key binding sites (red part) of the SH3 domain binding to its ligand (Fig. 4(a)), corresponding to the number of molecules in cluster reached about 225 (Fig. 4(e)). In the beginning, all nitrogen molecules dispersed in solution and collided randomly with the SH3 domain. Along with the aggregation of nitrogen molecules, the interface area caused by randomly collision decreased quickly and the interface area caused by cluster increased. As a result, in the first 10 ns, the interface area between nitrogen molecule and the SH3 domain rose first and then dropped down to about 3 nm^2. This is caused by the competition between the interface area caused by dispersed molecules and the interface area caused by cluster. The nitrogen cluster adsorbed on the SH3 domain until the 90 ns. In the next 80 ns, the cluster adsorbed on the SH3 domain, corresponding to a small fluctuation of interface area around 3 nm^2. Then, the cluster departed from the SH3 domain until the end of simulation. Meanwhile, the interface area dropped to about 0.5 nm^2.

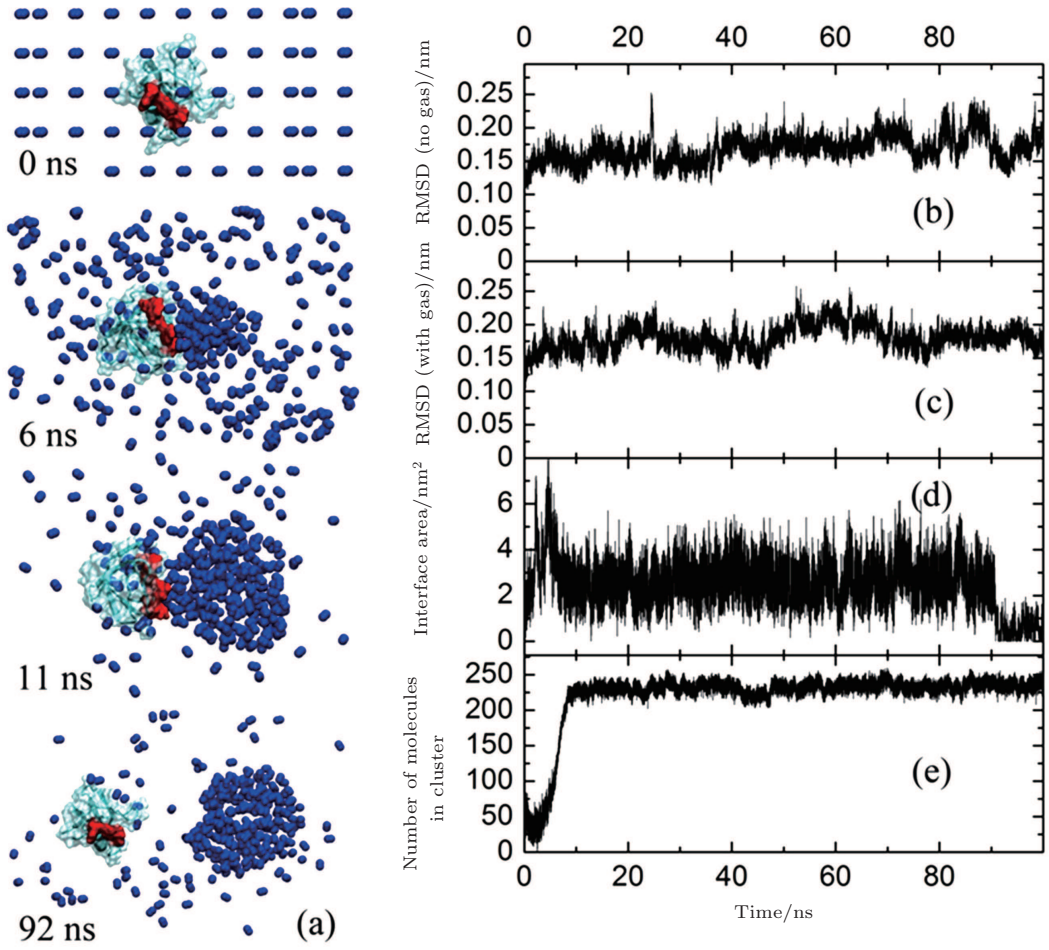


Fig. 4. The influences of SH3 when it binds to nitrogen cluster. (a)Some represented snapshots of simulation. (b) The RMSD of the SH3 domain in a system without nitrogen molecules (as control). (c) The RMSD of the SH3 domain in a system with nitrogen molecules, corresponding to Fig. 4(a). (d) The change of interface area between the nitrogen molecules and the SH3 domain. (e) The number of molecules in cluster for the system with nitrogen molecules.

We calculated the RMSD (root mean square deviation, Fig. 4(c), with gas) of the SH3 domain for this sample in the simulation to show the conformation change in simulation. The RMSD rose from 0 nm to 0.15 nm in very short time at the beginning of simulation, which was caused by the relaxation of protein in water. Then the RMSD fluctuated between 0.13 nm and 0.25 nm, no matter that the cluster adsorbed on the SH3 domain or departed from it. As control. Figure 4(b) shows the RMSD of another sample in which there were only SH3 domain and water (no gas). There is no significant difference between Figs. 4(b) and 4(c). This fact demonstrated that the adsorption of nitrogen cluster did not cause irreversible change to the conformation of the SH3 domain. It is reversible, which is in consistence with the requirement of anesthesia.

In our recent work, we calculated the nucleation concentration of inert gas molecules in water by molecular dynamic simulation. Our results indicated that the narcotic potency might be determined by the ratio of the solubility of inert gas in water under 1 atm (S), the nucleation concentration of inert gas (C), and the adsorption probability of protein to inert gas molecules (P) (Fig. 5). The simulated $(SP/C)_{\mathrm{R}}$ has a good linear correlation with the relative narcotic potency. The relative narcotic potencies of inert gases are obtained from experiments.[15] $(SP/C)_{\mathrm{R}}$ is the relative value of SP/C when we set SP/C of nitrogen as 1. Under this hypothesis, the steep dose relationship becomes understandable, due to the nucleation. In addition, our result also explains why different inert gases have different narcotic potencies. The C/S of neon is too large so that the partial pressure of neon in experiments hardly reaches the value needed by nucleation. The C/S of helium should be larger. Thus, the helium and neon usually are thought as non-anesthetics. It should be mentioned that the SH3 domain is not the binding target protein of anesthetics, so the P obtained from simulations might not be exactly consistent with the real value in the process of animal anesthesia. However, the SH3 domain is a good model to show the differences of hydrophobic affinity.

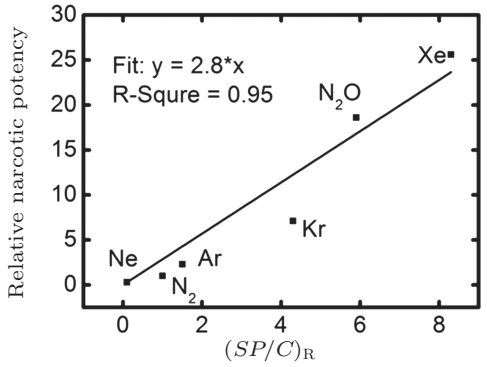


Fig. 5. The linear correlation between relative narcotic potency[15] and $(SP/C)_{\mathrm{R}}$.

5. Conclusions

The anesthetics have been used in clinic for more than a century. Until recent decades, the mechanisms of anesthesia have made great progress, although there are still problems hardly explained. We reviewed the history of anesthesia and the theories about general anesthesia, especially for inert gas molecules narcosis. We also proposed our hypothesis that the aggregated rather than the dispersed inert gas molecules are the causing factor to trigger the narcosis. By molecular dynamic simulation, we showed that the aggregated nitrogen molecules can adsorb on the hydrophobic area of the protein surface. In addition, if the inert gas molecules exert their effects as cluster state rather than dispersed molecules, some difficulties explained hardly by previous theories become understandable. The urgent need of our hypothesis is waiting for the direct experimental evidence. If it could be confirmed, it will enhance the safe application of inert gases in anesthesia and help to understand the molecular mechanism of anesthesia.

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References

[1] Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C and Alyautdin R 2002 *J. Drug Target.* **10** 317

[2] Nemmar A, Hoet P H M, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts M F, Vanbilloen H, Mortelmans L and Nemery B 2002 *Circulation* **105** 411

[3] Daniel M C and Astruc D 2004 *Chem. Rev.* **104** 293

[4] Michalet X, Pinaud F F, Bentolila L A, Tsay J M, Doose S, Li J J, Sundaresan G, Wu A M, Gambhir S S and Weiss S 2005 *Science* **307** 538

[5] Nel A, Xia T, Madler L and Li N 2006 *Science* **311** 622

[6] Rosi N L, Giljohann D A, Thaxton C S, Lytton-Jean A K R, Han M S and Mirkin C A 2006 *Science* **312** 1027

[7] Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G and Alexander A 2006 *Toxicol. Sci.* **92** 5

[8] Liu Z, Cai W B, He L N, Nakayama N, Chen K, Sun X M, Chen X Y and Dai H J 2007 *Nat. Nanotechnol.* **2** 47

[9] Gilbert N 2009 *Nature* **460** 937

[10] Hu W B, Peng C, Luo W J, Lv M, Li X M, Li D, Huang Q and Fan C H 2010 *ACS Nano* **4** 4317

[11] Zuo G H, Huang Q, Wei G H, Zhou R H and Fang H P 2010 *ACS Nano* **4** 7508

[12] Tu Y S, Lv M, Xiu P, Huynh T, Zhang M, Castelli M, Liu Z R, Huang Q, Fan C H, Fang H P and Zhou R H 2013 *Nat. Nanotechnol.* **8** 594

[13] Lacerda S H D, Park J J, Meuse C, Pristinski D, Becker M L, Karim A and Douglas J F 2010 *ACS Nano* **4** 365

[14] Moncada S, Palmer R M J and Higgs E A 1991 *Pharmacol. Rev.* **43** 109

[15] Bennett P B 2003 *Bennett and Elliott's Physiology and Medicine of Diving* (5th edn.) (London: Saunders) pp. 300–322

[16] Junod T 1835 *Ann. Gen. Med.* **9** 157

[17] Wells H 1847 *A History of the Discovery of the Application of Nitrous Oxide Gas, Ether and Other Vapors, to Surgical Operations*

[18] 1990 *The Elements* 71st (Boston: CRC Press) p. 4

[19] Cullen S C, Eger E I, Cullen B F and Gregory P 1969 *Anesthesiology* **31** 305

[20] Goto T, Suwa K, Uezono S, Ichinose F, Uchiyama M and Morita S 1998 *Br. J. Anaesth.* **80** 255

[21] Lawrence J, Loomis W, Tobias C and Turpin F 1946 *J. Physiol.* **105** 197

[22] Cullen S C and Gross E G 1951 *Science* **113** 580

[23] Lane G A, Nahrwold M L, Tait A R, Taylorbusch M, Cohen P J and Beaudoin A R 1980 *Science* **210** 899

[24] Eger E I, Saidman L J and Brandsta B 1965 *Anesthesiology* **26** 756

[25] Harless E and Bibra E V 1847 *Die Ergebnisse Der Versuche ber Die Wirkung Des Schwefeläthers (Titles in German)*

[26] Meyer H 1899 *Archiv für experimentelle Pathologie und Pharmakologie* **42** 109

[27] Meyer H 1899 *Archiv für experimentelle Pathologie und Pharmakologie* **42** 108

[28] Meyer H 1901 *Archiv für experimentelle Pathologie und Pharmakologie* **46** 338

[29] Overton C E 1901 *Studien ber Die Narkose Zugleich Ein Beitrag Zur Allgemeinen Pharmakologie*: Fischer) p. 195

[30] Skou J C 1958 *Biochim. Biophys. Acta* **30** 625

[31] Clements J A and Wilson K M 1962 *Proc. Natl. Acad. Sci. USA* **48** 1008

[32] Metcalfe J C, Seeman P and Burgen A S V 1968 *Mol. Pharmacol.* **4** 87

[33] Hill M W 1974 *Biochim. Biophys. Acta* **356** 117

[34] Mountcastle D, Biltonen R and Halsey M 1978 *Proc. Natl. Acad. Sci. USA* **75** 4906

[35] Bangham A D, Standish M M and Miller N 1965 *Nature* **208** 1295

[36] Franks N P and Lieb W R 1978 *Nature* **274** 339

[37] Eger E I, Saidman L J and Brandstater B 1965 *Anesthesiology* **26** 764

[38] Franks N and Lieb W 1996 *Anesthesiology* **84** 716

[39] Franks N and Lieb W 1984 *Nature* **310** 599

[40] Franks N P and Lieb W R 1994 *Nature* **367** 607

[41] 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 and Harrison N L 1997 *Nature* **389** 385

[42] Franks N P, Dickinson R, de Sousa S L M, Hall A C and Lieb W R 1998 *Nature* **396** 324

[43] Franks N P 2008 *Nat. Rev. Neurosci.* **9** 370

[44] Lou S T, Ouyang Z Q, Zhang Y, Li X J, Hu J, Li M Q and Yang F J 2000 *J. Vac. Sci. Technol. B* **18** 2573

[45] Ishida N, Inoue T, Miyahara M and Higashitani K 2000 *Langmuir* **16** 6377

[46] Holmberg M, Kuhle A, Garnaes J, Morch K A and Boisen A 2003 *Langmuir* **19** 10510

[47] Tyrrell J W G and Attard P 2001 *Phys. Rev. Lett.* **87** 176104

[48] Attard P 2003 *Adv. Colloid Interface Sci.* **104** 75

[49] Zhang X H, Zhang X D, Lou S T, Zhang Z X, Sun J L and Hu J 2004 *Langmuir* **20** 3813

[50] Dammer S M and Lohse D 2006 *Phys. Rev. Lett.* **96** 206101

[51] Smeets R M M, Keyser U F, Wu M Y, Dekker N H and Dekker C 2006 *Phys. Rev. Lett.* **97** 088101

[52] Zhang L, Zhang Y, Zhang X, Li Z, Shen G, Ye M, Fan C, Fang H and Hu J 2006 *Langmuir* **22** 8109

[53] Zhang X H, Maeda N and Craig V S J 2006 *Langmuir* **22** 5025

[54] Zhang X H, Khan A and Ducker W A 2007 *Phys. Rev. Lett.* **98** 136101

[55] Matsumoto M and Tanaka K 2008 *Fluid Dyn. Res.* **40** 546

[56] Ducker W A 2009 *Langmuir* **25** 8907

[57] Brenner M P and Lohse D 2008 *Phys. Rev. Lett.* **101** 214505

[58] Fang H and Hu J 2006 *Nucl. Sci. Tech.* **17** 71

[59] Wang C L, Li Z X, Li J Y, Xiu P, Hu J and Fang H P 2008 *Chin. Phys. B* **17** 2646

[60] Zhang L, Chen H, Li Z, Fang H and Hu J 2008 *Sci. China Ser. G* **51** 219

[61] Zhang M, Zuo G, Chen J, Gao Y and Fang H 2013 *Sci. Rep.* **3** 1660

[62] Koch C A, Anderson D, Moran M F, Ellis C and Pawson T 1991 *Science* **252** 668

[63] Pawson T and Gish G D 1992 *Cell* **71** 359

[64] Wu X D, Knudsen B, Feller S M, Zheng J, Sali A, Cowburn D, Hanafusa H and Kuriyan J 1995 *Structure* **3** 215

[65] Ball L J, Kuhne R, Schneider-Mergener J and Oschkinat H 2005 *Angew. Chem.-Int. Edit.* **44** 2852