

# X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel

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General anaesthetics have enjoyed long and widespread use but their molecular mechanism of action remains poorly understood. There is good evidence that their principal targets are pentameric ligand-gated ion channels<sup>1,2</sup> (pLGICs) such as inhibitory GABA<sub>A</sub> ( $\gamma$ -aminobutyric acid) receptors and excitatory nicotinic acetylcholine receptors, which are respectively potentiated and inhibited by general anaesthetics. The bacterial homologue from *Gloeobacter violaceus*<sup>3</sup> (GLIC), whose X-ray structure was recently solved<sup>4,5</sup>, is also sensitive to clinical concentrations of general anaesthetics<sup>6</sup>. Here we describe the crystal structures of the complexes propofol/GLIC and desflurane/GLIC. These reveal a common general-anaesthetic binding site, which pre-exists in the apo-structure in the upper part of the transmembrane domain of each protomer. Both molecules establish van der Waals interactions with the protein; propofol binds at the entrance of the cavity whereas the smaller, more flexible, desflurane binds deeper inside. Mutations of some amino acids lining the binding site profoundly alter the ionic response of GLIC to protons, and affect its general-anaesthetic pharmacology. Molecular dynamics simulations, performed on the wild type (WT) and two GLIC mutants, highlight differences in mobility of propofol in its binding site and help to explain these effects. These data provide a novel structural framework for the design of general anaesthetics and of allosteric modulators of brain pLGICs.

Understanding the mechanism of action of general anaesthetics requires the identification of their binding site(s) within the three-dimensional structure of pLGICs. To identify such a site, we used the pH-gated bacterial homologue GLIC, a homopentameric member of the pLGIC family that was recently shown to be sensitive to general anaesthetics<sup>6</sup> and amenable to X-ray structure determination<sup>4,5</sup>.

Co-crystals of GLIC were grown with propofol and apo-GLIC crystals were equilibrated in mother liquor saturated with desflurane. Diffraction data were collected up to 3.2-Å (desflurane) or 3.3-Å (propofol) resolution. In both structures, strong densities in otherwise empty Fourier  $F_o - F_c$  difference maps revealed bound anaesthetics in each subunit (mean peak height  $8.8 \pm 0.6\sigma$  for desflurane and  $5.9 \pm 0.8\sigma$  for propofol; Supplementary Fig. 1). Both molecules were found to bind in the same region, with little change in the protein conformation compared with apo-GLIC<sup>4</sup>, a feature observed also for some soluble proteins complexed with general anaesthetics<sup>7-9</sup>. The binding site is located in the upper half of the transmembrane domain (Fig. 1a) in a cavity that exists within each subunit of the apo-structure. The general-anaesthetic cavity is accessible from the lipid bilayer and progressively narrows down towards the interior of the subunit (Fig. 1b, c). Another cavity of comparable volume is located at the interface between subunits, on the other side of M1. It is not accessible from the outside. A narrow tunnel (less than 3 Å in diameter) links both cavities.

Residues from a single subunit border the general-anaesthetic cavity (Fig. 2), with contributions from M1 (I201, I202, M205 and L206), M2

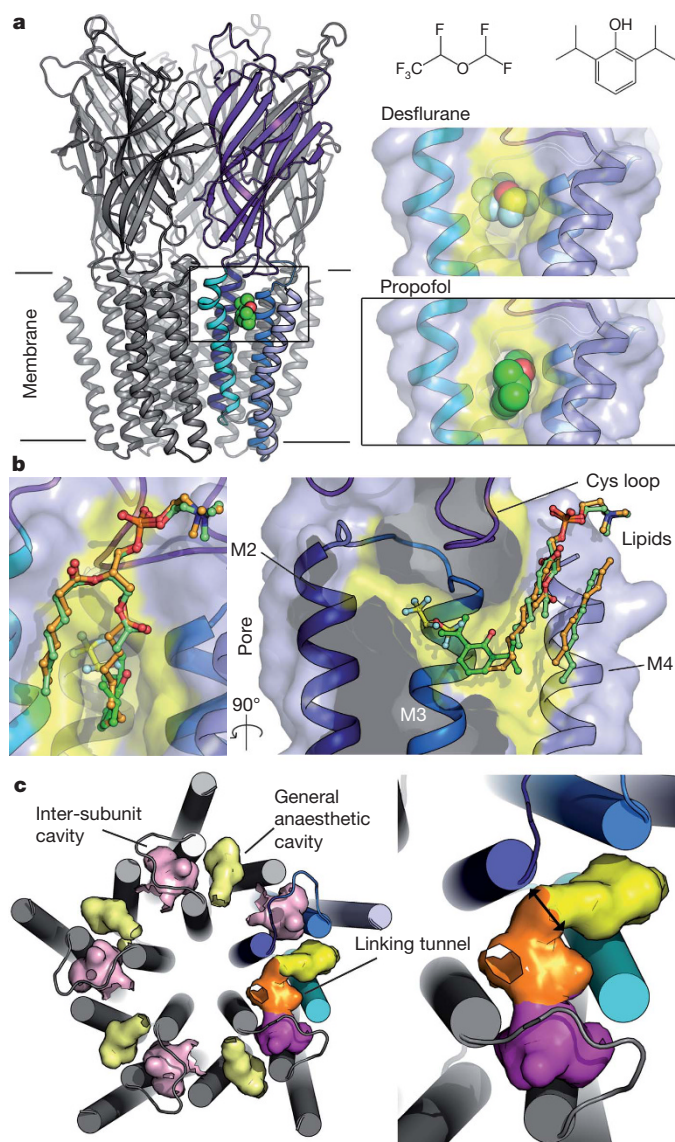
(the back wall, V242), M3 (Y254, T255, I258 and I259), M4 (N307 and F303, near the mouth), and from the  $\beta_6$ - $\beta_7$  loop (Y119, P120, F121, constituting the roof). Desflurane is buried deep inside the cavity and is engaged in mainly hydrophobic interactions with M1 (I201, I202), M3 (T255 and I258) and M2 (V242). Its oxygen atom is within hydrogen-bond distance of the T255 hydroxyl group. Significant additional electron density is observed in the protein neighbourhood and is attributed to lipids, with an alkyl chain obstructing the cavity entrance (Fig. 1b), as observed with the apo structure. This defines a cavity volume of  $238 \text{ \AA}^3$  whereas the volume of desflurane is  $94 \text{ \AA}^3$ . Propofol lies closer to the entrance of the general-anaesthetic cavity and would clash with the lipid seen in the apo and desflurane structures. Accordingly, the presence of propofol is associated with local acyl chain reorganization. Propofol is sandwiched between M1 and M3 and interacts mainly with T255 and Y254, by van der Waals contacts (Fig. 2). In the orientations that best fit the density maps, the propofol hydroxyl group could form a hydrogen bond with Y254. Propofol lies 6 Å away from the V242 side chain, whereas desflurane is 3.5 Å away.

We have recently shown that GLIC activation is inhibited by most general anaesthetics at clinical concentrations<sup>6</sup>. To check whether the general-anaesthetic binding sites contribute to this inhibition, we mutated key general anaesthetics-binding residues into alanine, or into more bulky residues (mutants I202A,W,Y, V242M,W and T255A), and studied the functional effect by two-electrode voltage-clamp electrophysiology in oocytes.

Among the mutants tested, I202Y and T255A produce a marked gain of function, with a tenfold shift of the proton dose-response curve towards lower concentrations ( $\text{pH}_{50} = 6.1 \pm 0.1$  with Hill number ( $n_H$ ) =  $2.0 \pm 0.2$  and  $\text{pH}_{50} = 6.0 \pm 0.2$  with  $n_H = 1.1 \pm 0.2$  respectively), compared with WT ( $\text{pH}_{50} = 5.0 \pm 0.3$  with  $n_H = 1.8 \pm 0.3$ ) (Fig. 3a, b). T255A shows also slower apparent rate constants for activation and deactivation. The other mutants are activated by protons in a manner similar to WT, except that V242W yielded no current (Supplementary Table 2).

The inhibitory action of general anaesthetics was measured around the one-fifth maximum effective concentration ( $\text{EC}_{20}$ ) of proton activation. On WT, propofol and desflurane produced 100% maximal inhibition with half-maximum inhibitory concentration ( $\text{IC}_{50}$ ) values of  $24 \pm 6.3 \mu\text{M}$  ( $n_H = 1.1 \pm 0.2$ ) and  $27 \pm 13 \mu\text{M}$  ( $n_H = 0.3 \pm 0.2$ ) respectively. Screening the mutants for inhibition by 10  $\mu\text{M}$  propofol and 500  $\mu\text{M}$  desflurane shows no change when position 202 is mutated, but shows that V242M and T255A produce a parallel tenfold shift of the propofol dose-inhibition curve to lower concentration (Fig. 3c, d and Supplementary Table 3). In contrast, V242M has no effect on desflurane inhibition, whereas T255A produces a tenfold shift of the desflurane inhibition curve towards higher concentrations. Measurement of general-anaesthetic inhibition at different pH shows that both anaesthetics are more efficient at higher pH, for both WT and T255A (Fig. 3e and Supplementary Fig. 2). Strikingly, T255A increases the

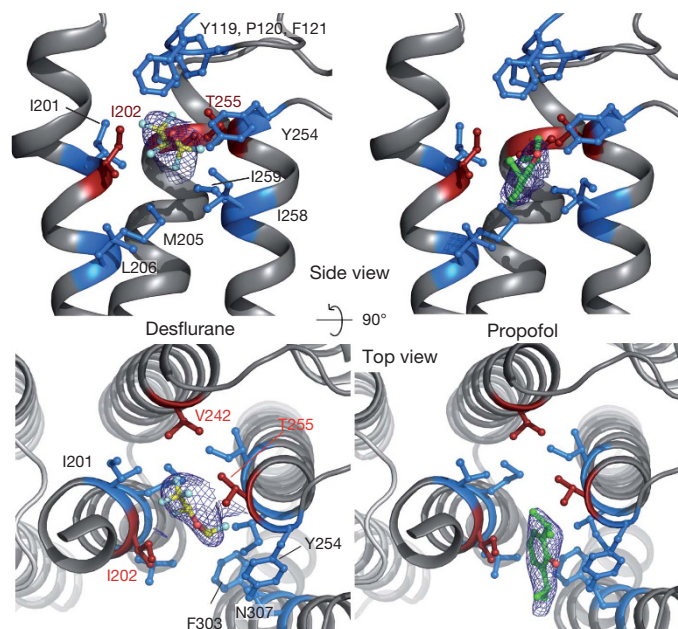
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**Figure 1 | Propofol and desflurane binding sites.** **a**, General view of GLIC from the plane of the membrane in cartoon representation with a bound general-anaesthetic molecule in space-filling representation. The molecular surface is represented in the insets and coloured in yellow for the binding pocket. **b**, Cartoon and surface representation of the general-anaesthetic cavity seen from the membrane (left) and from the adjacent subunit (right, M1 removed for clarity) with propofol (green), desflurane (yellow) and lipids of the two structures (green and orange respectively) depicted as sticks. For this representation C $\alpha$  atoms were superimposed with a root mean square deviation of 0.13 Å. **c**, Molecular surface of the general-anaesthetic intra-subunit cavities (yellow) and neighbouring inter-subunit cavities (pink) for the whole pentamer. In one of the subunits, the communication tunnel between the two cavities is depicted in orange, and its constriction indicated by an arrow in the inset.

inhibition by propofol but decreases the inhibition by desflurane at all proton concentrations. Altogether, mutation of selected residues within the general-anaesthetic binding site affects (1) the intrinsic ionic response of GLIC, illustrated by the marked gain of function of I202Y and T255A, whose phenotypes are similar to that of the canonical I233(9')A mutation<sup>3,10</sup>, and (2) the pharmacology of general anaesthetics, illustrated both by V242M, which displays an increased sensitivity to propofol but not to desflurane, and T255A, which has an increased sensitivity to propofol but a decreased sensitivity to desflurane.

These data support the hypothesis that the general-anaesthetic binding site described here contributes to general-anaesthetic-mediated

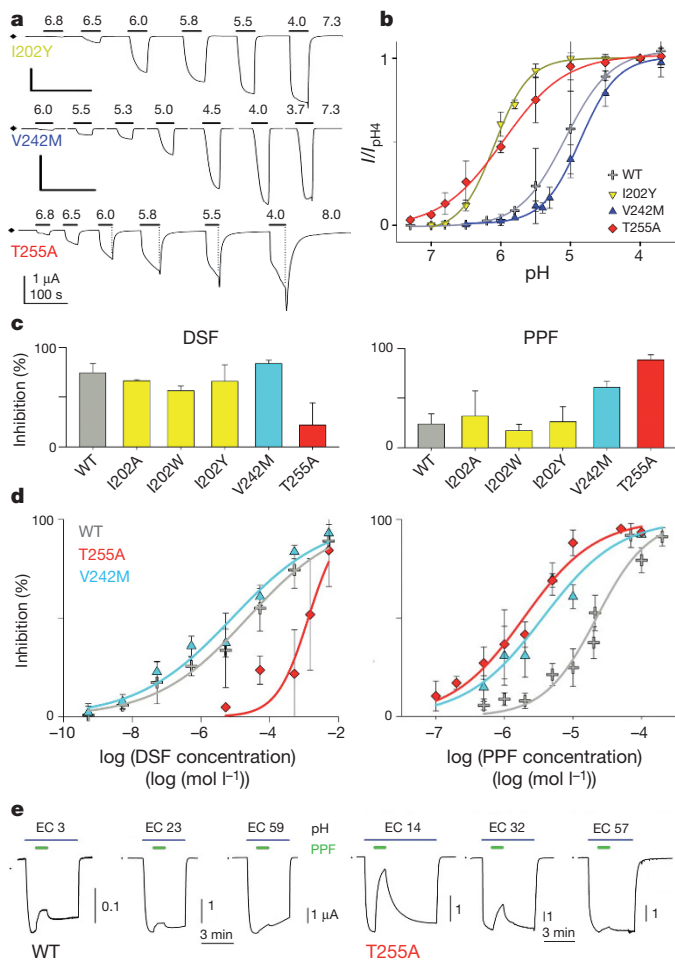


**Figure 2 | Residues of the binding site.** Sites for propofol (right) and desflurane (left), viewed from the membrane (top panels with M4 helix removed), and from the ECD domain (lower panels with ECD removed). Residues bordering the pocket and contributing to binding are depicted as blue or red (mutated positions) sticks. SigmaA weighted Fourier difference maps  $2F_o - F_c$  contoured at  $1.5\sigma$  around the anaesthetics molecules are represented as a blue mesh.

inhibition of GLIC. For desflurane, mutagenesis data match the characteristics of its binding site in the X-ray structure well, with no significant effect when the relatively distant positions 202 and 242 are mutated, and a strong impairing effect when mutating T255, which extensively contacts desflurane, into an alanine. In contrast, for propofol, both positions 202 and 255 contact propofol, but only mutation at position 255 alters its effect. More surprisingly, position 242 is not in direct contact with propofol but V242M modifies its response. These data suggest a significant mobility of propofol within the cavity, a feature that may be reflected by the high  $B$  factors of general anaesthetics in the crystal structure ( $B_{\text{desflurane}} = 121 \text{ \AA}^2$ ,  $B_{\text{propofol}} = 135 \text{ \AA}^2$ , mean values), although high  $B$  factors and partial occupancy of the site cannot be discriminated at 3.3-Å resolution.

To examine this possibility further, we performed 30-ns molecular dynamics simulations of propofol bound to the WT protein, T255A, V242M and I202A mutants. At this timescale, propofol remains in the cavity, but shows substantial mobility (Fig. 4a). T255A and V242M are associated with (1) reduced propofol fluctuation (root mean square fluctuation of propofol non-H atoms of  $3 \pm 1.1 \text{ \AA}$ ,  $2.4 \pm 0.8 \text{ \AA}$ ,  $2.3 \pm 0.8 \text{ \AA}$ ,  $2.7 \text{ \AA}$  for the WT, T255A, V242M and I202A runs, respectively), (2) deeper penetration inside the cavity (Fig. 4b) and (3) more frequent interaction with residue 242 compared with the WT and I202A (data not shown). Altogether, these simulations provide complementary interpretations to account for the higher sensitivity of T255A and V242M to propofol inhibition that could not have been deduced from the static structure alone.

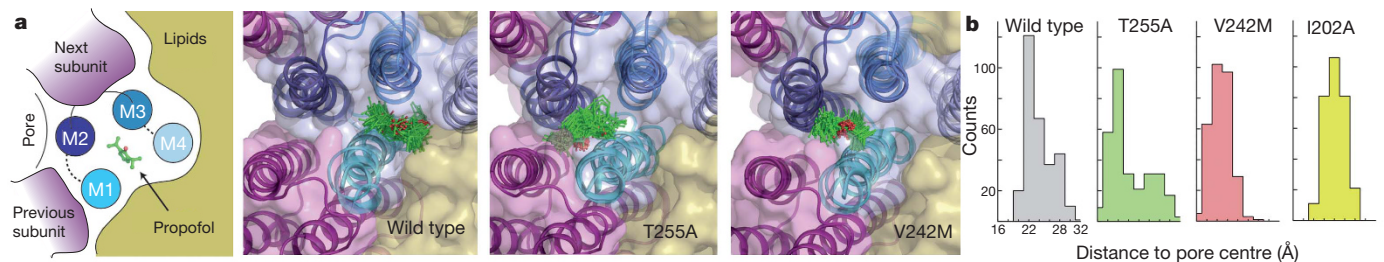
The X-ray structure of GLIC was formerly interpreted in terms of an apparently open conformation<sup>4,5</sup>. But general anaesthetics behave as inhibitors of the ionic response and are therefore expected to stabilize a closed conformation. Our data unravel a general-anaesthetic site in the open conformation, and molecular dynamics simulations show that propofol and desflurane are stable in this site conformation at the 30-ns timescale. This apparent contradiction can be readily explained by a non-exclusive (differential) binding of general anaesthetics to the open and closed states, with general anaesthetics displaying a higher affinity for the closed state than for the open one<sup>11</sup>. Interestingly, the T255A and I202Y gain-of-function phenotypes suggest a structural rearrangement



**Figure 3 | Electrophysiological characterization of binding-site residues.**

**a**, Traces of currents evoked by 30-s applications of low extracellular pH separated by 30–60 s wash. **b**, Corresponding plots for currents normalized with respect to the value at pH 4. Mean  $\pm$  s.d. of 4 to 12 cells per construct. **c**, Inhibition by 0.5 mM desflurane (left) or 10  $\mu$ M propofol (right) applied for 60 s during the plateau of GLIC activation by a pH near EC<sub>20</sub> (EC<sub>10–30</sub>). **d**, Corresponding concentration–inhibition characteristics of desflurane (left) or propofol (right). **e**, Current traces showing the effect of 10  $\mu$ M propofol on GLIC currents corresponding to proton EC<sub>3,23,59</sub> (WT, left traces) or EC<sub>14,32,57</sub> (T255A, right traces) of each cell. *Xenopus laevis* oocytes, holding potential –60 to –40 mV.

of the general-anaesthetic binding site during gating, in line with its location on the backside of the pore-lining M2 helices, at the level of the gate, and close to the transmembrane-domain–extracellular-domain interface. We note that the hypothetical gating mechanism<sup>4</sup> previously suggested from the comparison of GLIC and ELIC<sup>12</sup> structures involves



**Figure 4 | Molecular dynamics simulation of propofol bound to GLIC.**

**a**, View from the ECD domain depicting propofol positions as green sticks, superposed at 0.5-ns intervals onto the starting (cartoon and molecular surface) and final (transparent cartoon) conformation of the protein, during the 30-ns molecular dynamics runs. The scheme on the left explains the colour code, and

a strong reorganization of the general-anaesthetic binding site as a consequence of M2 and M3 helix tilting.

Another important feature of the GLIC–general anaesthetic structures is that binding occurs to a site where nearby ordered lipids are identified. One lipid lying in the crevice between M1 and M4 is observed in the apo and desflurane structures and is displaced in the propofol structure. These lipids co-purify with the solubilized protein, indicating tight binding within protein subsites known to be critical for the transmembrane domain structure<sup>13,14</sup>. It is known that lipids contribute to pLGIC function<sup>15,16</sup>, and those observed in the present electron-density maps are good candidates for such a role. A perturbation of interactions with lipids caused by general-anaesthetic binding might thus contribute to the functional inhibition, suggesting that general anaesthetics may compete with endogenous allosteric modulators<sup>17</sup>, lipids in this case, but also possibly fatty acids, cholesterol and/or neurosteroids<sup>18</sup> in the case of eukaryotic pLGICs.

It is striking that the pharmacology of GLIC inhibition resembles that of nicotinic acetylcholine receptors (nAChRs), which are also inhibited by general anaesthetics and are unusually sensitive to volatile anaesthetics<sup>19</sup>. The general-anaesthetic binding site described here is thus a primary candidate for promoting nAChR inhibition. In contrast, Gly/GABA<sub>A</sub> receptors are mostly potentiated by general anaesthetics. Experimental data involving chimaeric constructs show that GlyR $\alpha$ 1 S267 (M2), A288 (M3) and I229 (M1) contribute to general anaesthetic and alcohols potentiation<sup>2,20</sup>. The general anaesthetic etomidate labelled brain GABA<sub>A</sub> receptors at residues  $\alpha$ 1M236 and  $\alpha$ 3M286<sup>21</sup>, the latter corresponding to GlyR $\alpha$ 1 A288. This labelling was only partly inhibited by propofol and neurosteroids<sup>22</sup>, consistent with an allosteric interaction between several binding sites. Overall, the interpretation of these data using homology models based on the cryoelectron-microscopy nAChR structure at medium resolution<sup>23–25</sup> suggests that intra-subunit and/or inter-subunit sites in the upper part of the transmembrane domain mediate general-anaesthetic modulation of anionic pLGICs.

The sequence of GLIC can be readily aligned with that of GABA<sub>A</sub>R and GlyR (Supplementary Fig. 3). From the GLIC three-dimensional structure and this alignment, the three residues identified in GABA<sub>A</sub>/Gly receptors can be seen to point towards the inter-subunit cavity (Fig. 1c) close to the intra-subunit general-anaesthetic binding site described here. A marked reorganization of these cavities was observed in the initial steps of channel closing during our 1- $\mu$ s molecular dynamics simulation of GLIC at neutral pH<sup>26</sup> (Supplementary Fig. 4). This involves transient communications between the inter- and intra-subunit cavities caused by M2 and M3 motions, which further supports the notion that the shape and volume of the cavity are coupled to channel gating, and could suggest that a cross-talk between both cavities might underlie general-anaesthetic-mediated potentiation.

pLGIC and particularly nAChRs are not only the target of general anaesthetics<sup>27</sup> but also of natural and synthetic allosteric modulators that are developed for their therapeutic potential<sup>28,29</sup>. Mutational data suggest that ivermectine<sup>30</sup> and PNU-120596, which behave as positive

the three panels correspond to the WT, T255A and V242M systems. **b**, Distance distribution between the propofol centre of mass and the pore centre. The distribution is shifted closer to the pore centre for the mutants showing an increased inhibition.

allosteric modulators of  $\alpha 7$  nAChR, bind at a location resembling that observed in our structures. The general-anaesthetic binding site unravelled herein thus provides a novel template for the design of allosteric modulators inhibiting or potentiating pLGICs.

## METHODS SUMMARY

GLIC production<sup>4</sup>, electrophysiology<sup>3</sup> and molecular dynamics<sup>26</sup> were performed as described (full methods in the Supplementary Information). Crystals were typically grown in 12–16% PEG 4000, 400 mM NaSCN, 100 mM Na-Acetate at pH 4, in the presence of an excess of general anaesthetics.

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Author Contributions** All authors contributed extensively to the work presented in this paper.

**Author Information** The coordinates of models are deposited in Protein Data Bank under accession numbers 3P50 (propofol) and 3P4W (desflurane). Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at [www.nature.com/nature](http://www.nature.com/nature). Correspondence and requests for materials should be addressed to P.-J.C. ([picorin@pasteur.fr](mailto:picorin@pasteur.fr)) or M.D. ([delarue@pasteur.fr](mailto:delarue@pasteur.fr)).

## METHODS

**Protein production.** The protein was produced and purified as described previously<sup>3,4,26</sup> with a few variations. The GLIC protein was overexpressed in *Escherichia coli* C43 cells, and expression was induced with 0.1 mM isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) at absorbance ( $A_{600\text{ nm}}$ ) = 1 overnight at 20 °C. Cells were mechanically lysed in buffer 1 (Tris 20 mM pH 7.6, NaCl 300 mM, with proteases inhibitors from Roche); membranes were isolated by ultracentrifugation. The proteins were extracted from membranes with 2% DDM (Anatrace) under agitation at 4 °C, and the solubilized fraction was cleared by ultracentrifugation. Solubilized proteins were first purified by affinity chromatography on an amylose resin. After extensive wash, in buffer 1 supplemented with 0.1% DDM, the MBP-GLIC fusion protein bound to the resin was cleaved overnight at 4 °C under gentle agitation, in the presence of three units of thrombin (Calbiochem) per 50  $\mu$ g of protein and of 2 mM of CaCl<sub>2</sub>. The digested protein was eluted in buffer 1 supplemented in 0.02% DDM and concentrated. It was then subjected to size exclusion chromatography on a Superose 6 10/300 GL column (GE Healthcare) equilibrated in the same buffer. Fractions of the peak corresponding to the pentamer were pooled and concentrated for crystallization experiments. **Crystallography.** GLIC was crystallized using the vapour diffusion method in hanging drops at 20 °C. The concentrated (8–12 mg ml<sup>-1</sup>) protein was mixed in a 1:1 ratio with reservoir solution containing typically 12–16% PEG 4000, 400 mM NaSCN and 0.1 M NaAc at pH 4.0. Crystals of the protein grew overnight. A saturating amount of desflurane was then added in the well (typically the well of a Linbro plate was completely filled). After 2–5 days of equilibration the crystals were rapidly transferred in the mother solution supplemented with 20% glycerol for cryoprotection and flash-frozen in liquid nitrogen. Pure propofol was added at the time of crystallization right after the mixture of the protein solution with the mother liquor. An emulsion was formed and small crystals grew in 2 days, nearby the propofol droplets included in the crystallization drops. Crystals were flash-frozen in the usual manner.

A great number of data sets of frozen single crystals were collected on beamlines Proxima-I of the Soleil Synchrotron and ID14 of the European Synchrotron Radiation Facility and processed with XDS<sup>31</sup> and CCP4 (ref. 32) programs. Crystals were isomorphous to the WT ones, with a C2 space group and one pentamer in the asymmetric unit. Non-crystallographic symmetry (NCS) restraints were used throughout the refinement performed by alternate cycles of manual building in COOT<sup>33</sup> and automatic refinement using REFMAC<sup>34</sup> and BUSTER<sup>35</sup>. Difference Fourier  $F_o - F_c$  maps were checked for strong signals indicating the presence of a ligand. In the two data sets presented here, bound anaesthetics corresponded to 5–9 $\sigma$  peaks (Supplementary Fig. 1), depending on subunits. The peaks were present in each subunit in a region devoid of any density in other data sets. Moreover, the electron density for the known bound detergents in the pore<sup>4</sup> appears below or at this level (data not shown), namely 6 $\sigma$  for the most ordered part of the detergent, which constitutes an intrinsic positive control for the presence of anaesthetics. At 3.2- to 3.3-Å resolution it may not be justified to model the bound molecules individually as this will result in a different orientation in each subunit. For propofol we used fivefold NCS-averaged maps to build the molecules. For desflurane we also used NCS maps; in addition, both plausible orientations were tried and the one with the trifluoromethyl group at the bottom of the cavity was selected by comparing difference maps after refinement and independent docking scores (data not shown).

Lipids surrounding the anaesthetics binding site were partly modelled, in NCS-averaged maps. Identification of the chemical nature of the lipids is not possible with such maps at this resolution and thus we arbitrarily used phosphatidylcholine. As in the apo- protein model, the acyl chains are more ordered than the polar heads. One small part of an acyl chain corresponding to a second layer of lipid with no direct interaction with the protein is present. Structural analysis and figure preparation were done with PyMOL<sup>36</sup>, VMD<sup>37</sup> and Molprobit<sup>38</sup>. Refinement parameters for ligands were generated with the PRODRG server<sup>39</sup>.

**Electrophysiology.** Conditions for electrophysiological experiments were as follows (apart from a few variations<sup>9</sup> in experiments using desflurane).

**Cell injection.** Defolliculated, stage VI<sup>40</sup> *X. laevis* oocytes were obtained from a commercial supplier one day after ovary dissection. DNA (<2 ng) was blind injected into the nucleus through the animal pole, using a pneumatic microinjector, as a mixture in water of GLIC cDNA in a pmt3 vector (0.08 g l<sup>-1</sup>) and green fluorescent protein (GFP) cDNA in the same vector (0.02 g l<sup>-1</sup>), as a reporter gene for successful intranuclear injection. Identified cells were kept in 96-well plates with U-shaped bottom, in a HEPES-buffered modified Barth's<sup>41</sup> solution (in mM: NaCl 88, KCl 1, NaHCO<sub>3</sub> 2.4, HEPES 20, MgSO<sub>4</sub> 0.82, Ca(NO<sub>3</sub>)<sub>2</sub> 0.33, CaCl<sub>2</sub> 0.41; pH 7.4; 0.22  $\mu$ m filtered), at 18 °C for two days and then at 15 °C. Oocytes with the T255A mutant were transferred to pH 8 Barth's solution 2 days after injection. GFP-positive oocytes selected 2 days after injection were recorded 2–6 days after injection.

**Electrophysiological recordings.** Oocytes<sup>42</sup> were superfused with the animal pole facing a gravity driven solution inflow (4–8 ml min<sup>-1</sup>) in a corridor-shaped recording chamber (flow section <10 mm<sup>2</sup>). A solution of (in mM) NaCl 100, KCl 3, CaCl<sub>2</sub> 1,

MgCl<sub>2</sub> 1 and 2-morpholino-ethanesulphonic acid (MES) 10 was adjusted to pH 8.0 with NaOH, and used as intertest on T255A oocytes. The control extracellular pH (7.3) and lower pH values were reached by adding HCl 2 mol l<sup>-1</sup> and any extra pH 8.0 solution. The whole oocyte plasma membrane was voltage clamped (GeneClamp 500, Axon Instruments/Molecular Devices) using two intracellular pipettes filled with 3 M KCl (0.8–1.5 M $\Omega$ ), and distinct current and voltage extracellular electrodes separately bridged to the bath near suction using 5 g l<sup>-1</sup> agar in 3 M KCl. Currents were recorded (pClamp8, Axon Instruments) at air-conditioned room temperature (21–23 °C), acquired at 500 Hz after low-pass filtering (200 Hz), and further filtered using 100- to 1-mean sample data reduction for figure display. Proton concentration–response curves were established at a holding potential of –50 mV, using manually controlled 30-s test-pH applications (or shorter when desensitization/inhibition at low pH produced a peak current) separated by 30- to 60-s wash at pH 7.3, or 8.0 for the T255A GLIC mutant.

**Preparation of general-anaesthetic solutions.** Desflurane was obtained from Baxter Healthcare Corporation. Desflurane solutions were made up gravimetrically, in gas-tight ground-glass syringes, and vigorously agitated. The final concentration was spot-checked by headspace gas chromatography. Propofol (2,6 diisopropylphenol) was obtained from Aldrich (W50,510-2). Propofol stock solution was made by dissolving pure oily liquid propofol at 1 mol l<sup>-1</sup> in dimethyl sulphoxide (DMSO). It was kept in glass in the dark at room temperature for up to 1 month, and diluted in the recording solution less than 2 h before use on each cell, under strong agitation to 0.1 mM and then to lower concentrations. For most of the recordings, a single concentration of propofol was present in the perfusion system, and each cell received a single application of propofol. The perfusion system was extensively cleaned and partly replaced before going from high to low propofol concentrations.

**Fit of data and statistics.** Data in Fig. 3b–e are presented as mean  $\pm$  s.d. Plots shown in Fig. 3b, d of log(concentration/mol l<sup>-1</sup>) versus mean effect were fitted with a sigmoid function. Parameters given in Supplementary Table 2, and in Supplementary Table 3 for desflurane, were obtained by fitting for each cell a plot of concentration/mol l<sup>-1</sup> versus normalized effect over four or five orders of magnitude with the Hill equation, giving values of  $n_H$  and half-maximum effective concentration (EC<sub>50</sub>) (proton) or IC<sub>50</sub> (desflurane) for individual cells, of which mean  $\pm$  s.d. values are shown. Parameters given in Supplementary Table 3 for propofol were obtained by Hill-fitting a scatter plot of individual concentration/mol l<sup>-1</sup> versus percentage inhibition data points obtained from all the cells tested (one data point per cell in most cases);  $n_H$  and IC<sub>50</sub> are given with the standard error of the parameters determined from the nonlinear regression. Data in Supplementary Fig. 2 were empirically fitted with a straight line, or a simple exponential decay, to improve readability.

**Molecular dynamics.** We used a full atomic model of GLIC at pH 4.6 derived from previous simulations<sup>26</sup> to add general-anaesthetic molecules at the positions determined in the crystal structures presented in this work. The protonation state was assigned similar to previous simulations on the basis of pK<sub>a</sub> calculations with the Yasara software<sup>43</sup> to represent the most probable pattern at pH 4.6, with residues E26, E35, E67, E75, E82, D86, D88, E177 and E243 being protonated. H277 was doubly protonated. The model was inserted in a fully hydrated palmitoyl-2-oleoyl-sn-glycerol-phosphatidylcholine (POPC) lipid bilayer (307 lipids, approximately 44,000 water molecules) leading to an initial system size of 128 Å  $\times$  125 Å  $\times$  182 Å. The net charge of the system was neutralized with 54 Na<sup>+</sup> and 89 Cl<sup>-</sup> counterions, achieving a salt concentration of about 100 mM. These steps were performed within VMD<sup>37</sup>, using the psfgen, membrane, solvate and autoionize plug-ins. Similar models were derived for the I202A, V242M and T255A mutants. The simulations were performed with NAMD<sup>44</sup> using the CHARMM27 (ref. 45) force field. Parameters for propofol were provided by G. Brannigan<sup>9</sup>.

A short equilibration was performed by minimizing the system for 1,000 steps. This was followed by 30 ns of production runs. Simulations were performed at 310 K using Langevin dynamics with a damping coefficient  $\gamma$  of 1 ps<sup>-1</sup> for temperature control. A Langevin piston algorithm was used to maintain the pressure at 1 atm. A short 10-Å cutoff was used for non-bonded interactions. A smooth switching function was used for van der Waals interactions between 8.5 and 10 Å. Long-range electrostatic interactions were treated using the particle mesh Ewald method<sup>46</sup>. The r-RESPA multiple time step method<sup>47</sup> was used with a 2-fs time step for bonded and for short-range non-bonded interactions, and a 4-fs step for long-range electrostatic forces. All bonds between hydrogen atoms and heavy atoms were constrained with the SHAKE algorithm. All molecular dynamics simulations were performed on Vargas, an IBM Regatta Power6 machine at the Institut du Développement et des Ressources en Informatique Scientifique (IDRIS) Supercomputer Center in Orsay (France).

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