Intracellular Transfer of Na+ in an Active State G Protein Coupled Receptor

Vickery, Owen; Carvalheda Dos Santos, Catarina; Zaidi, Saheem A.; Pisliakov, Andrei; Katritch, Vsevolod; Zachariae, Ulrich

Published in:
Structure

DOI:
10.1016/j.str.2017.11.013

Publication date:
2018

Document Version
Peer reviewed version

Intracellular Transfer of Na^+ in an Active State G-Protein Coupled Receptor

Owen N. Vickery[1,2], Catarina A. Carvalheda[1,2], Saheem A. Zaidi[3], Andrei V. Pisliakov[1,2], Vsevolod Katritch[3,4], Ulrich Zachariae[1,2,*]

[1]School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

[2]School of Science and Engineering, University of Dundee DD1 4NH, UK.

[3]Departments of Biological Sciences and [4]Chemistry, Bridge Institute, University of Southern California, Los Angeles, CA 90089, USA

*Corresponding author; email: u.zachariae@dundee.ac.uk

*Lead Contact; email: u.zachariae@dundee.ac.uk

ABSTRACT

Playing a central role in cell signaling, GPCRs are the largest superfamily of membrane proteins and form the majority of drug targets in humans. How extracellular agonist binding triggers the activation of GPCRs and associated intracellular effector proteins remains, however, poorly understood. Structural studies have revealed that inactive class-A GPCRs harbor a conserved binding site for Na^+ ions in the center of their transmembrane domain, accessible from the extracellular space. Here, we show that the opening of a conserved hydrated channel in the activated state receptors allows the Na^+ ion to egress from its binding site into the cytosol. Coupled with protonation changes, this ion movement occurs without significant energy barriers, and can be driven by physiological transmembrane ion and voltage gradients. We propose that Na^+ ion exchange with the cytosol is a key step in GPCR activation. Further, we hypothesize that this transition locks receptors in long-lived active-state conformations.

INTRODUCTION

G-protein coupled receptors (GPCRs) mediate the transfer of external ligand binding information across the plasma membrane to activate a range of intracellular signaling pathways **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nrm908", "ISBN" : "1471-0072 (Print)\\n1471-0072 (Linking)", "ISSN" : "14710072", "PMID" : "12209124", "abstract" : "Seven-transmembrane receptors, which constitute the largest, most ubiquitous and most versatile family of membrane receptors, are also the most common target of therapeutic drugs. Recent findings indicate that the classical models of G-protein coupling and activation of second-messenger-generating enzymes do not fully explain their remarkably diverse biological actions.", "author" : [{ "dropping-particle" : "", "family" : "Pierce", "given" : "Kristen L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Premont", "given" : "Richard T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lefkowitz", "given" : "Robert J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature reviews. Molecular cell biology", "id" : "ITEM-1", "issue" : "September", "issued" : { "date-parts" : [["2002"]] }, "page" : "639-650", "title" : "Seven-transmembrane receptors.", "type" : "article-journal", "volume" : "3" }, "uris" : ["http://www.mendeley.com/documents/?uuid=203182a1-3741-46b1-8cc5-9251b9c7c01b"] }], "mendeley" : { "formattedCitation" : "(Pierce et al., 2002)", "plainTextFormattedCitation" : "(Pierce et al., 2002)", "previouslyFormattedCitation" : "(Pierce et al., 2002)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Playing a central role in regulation of vital biological systems, including nervous, cardiovascular, immune, digestive, reproductive etc., they represent the majority of membrane proteins in humans and the largest class of present drug targets **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nrd2199", "ISSN" : "1474-1776", "PMID" : "17139284", "abstract" : "For the past decade, the number of molecular targets for approved drugs has been debated. Here, we reconcile apparently contradictory previous reports into a comprehensive survey, and propose a consensus number of current drug targets for all classes of approved therapeutic drugs. One striking feature is the relatively constant historical rate of target innovation (the rate at which drugs against new targets are launched); however, the rate of developing

drugs against new families is significantly lower. The recent approval of drugs that target protein kinases highlights two additional trends: an emerging realization of the importance of polypharmacology, and also the power of a gene-family-led approach in generating novel and important therapies.", "author" : [{ "dropping-particle" : "", "family" : "Overington", "given" : "John P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Al-Lazikani", "given" : "Bissan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hopkins", "given" : "Andrew L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature reviews. Drug discovery", "id" : "ITEM-1", "issue" : "12", "issued" : { "date-parts" : [["2006", "12"]] }, "page" : "993-6", "title" : "How many drug targets are there?", "type" : "article-journal", "volume" : "5" }, "uris" : ["http://www.mendeley.com/documents/?uuid=170677d0-c0cf-4c3d-8d34-a2a67abfa7df"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1146/annurev-pharmtox-011613-135943", "ISBN" : "1545-4304 (Electronic)\\n0362-1642 (Linking)", "ISSN" : "0362-1642", "PMID" : "24016212", "abstract" : "The largest innovations within pharmaceutical development come through new compounds that have unique and novel modes of action. These innovations commonly involve expanding the protein space targeted by pharmaceutical agents. At present, information about drugs and drug targets is available online via public databases such as DrugBank and the Therapeutic Targets Database. However, this information is biased, understandably so, toward established drugs and drug-target interactions. To gain a better overview of the drug-targeted portion of the human proteome and the directions of current drug development, we developed a data set of clinical trial drug-target interactions based on CenterWatch's Drugs in Clinical Trials Database, one of the largest databases of its kind. Our curation identified 475 potentially novel clinical trial drug targets. This review aims to identify trends in drug development based on the potentially novel targets currently being explored in clinical trials.", "author" : [{ "dropping-particle" : "", "family" : "Rask-Andersen", "given" : "Mathias", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Masuram", "given" : "Surendar", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schi\u00f6th", "given" : "Helgi B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Annual Review of Pharmacology and Toxicology", "id" : "ITEM-2", "issue" : "1", "issued" : { "date-parts" : [["2014", "1", "6"]] }, "page" : "9-26", "title" : "The Druggable Genome: Evaluation of Drug Targets in Clinical Trials Suggests Major

Shifts in Molecular Class and Indication", "type" : "article-journal", "volume" : "54" }, "uris" : ["http://www.mendeley.com/documents/?uuid=79521d08-913a-40be-8a49-60d5b3d128cd"] }], "mendeley" : { "formattedCitation" : "(Overington et al., 2006; Rask-Andersen et al., 2014)", "plainTextFormattedCitation" : "(Overington et al., 2006; Rask-Andersen et al., 2014)", "previouslyFormattedCitation" : "(Overington et al., 2006; Rask-Andersen et al., 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . In recent years, a number of crystal structures have been solved to reveal conformational changes between inactive and active state receptors, including common movement in transmembrane helices and conserved microswitches **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature11896", "ISSN" : "1476-4687", "PMID" : "23407534", "abstract" : "G-protein-coupled receptors (GPCRs) are physiologically important membrane proteins that sense signalling molecules such as hormones and neurotransmitters, and are the targets of several prescribed drugs. Recent exciting developments are providing unprecedented insights into the structure and function of several medically important GPCRs. Here, through a systematic analysis of high-resolution GPCR structures, we uncover a conserved network of non-covalent contacts that defines the GPCR fold. Furthermore, our comparative analysis reveals characteristic features of ligand binding and conformational changes during receptor activation. A holistic understanding that integrates molecular and systems biology of GPCRs holds promise for new therapeutics and personalized medicine.", "author" : [{ "dropping-particle" : "", "family" : "Venkatakrishnan", "given" : "a J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Deupi", "given" : "Xavier", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lebon", "given" : "Guillaume", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tate", "given" : "Christopher G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schertler", "given" : "Gebhard F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Babu", "given" : "M Madan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7436", "issued" : { "date-parts" : [["2013", "3", "14"]] }, "page" : "185-94", "publisher" : "Nature Publishing Group", "title" : "Molecular signatures of G-protein-coupled receptors.", "type" : "article-journal", "volume" : "494" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=e5af2e56-b4fa-4c54-a944-40a93e05d3b6"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1146/annurev-pharmtox-032112-135923", "ISSN" : "1545-4304", "PMID" : "23140243", "abstract" : "During the past few years, crystallography of G protein-coupled receptors (GPCRs) has experienced exponential growth, resulting in the determination of the structures of 16 distinct receptors-9 of them in 2012 alone. Including closely related subtype homology models, this coverage amounts to approximately 12% of the human GPCR superfamily. The adrenergic, rhodopsin, and adenosine receptor systems are also described by agonist-bound active-state structures, including a structure of the receptor-G protein complex for the \u03b2(2)-adrenergic receptor. Biochemical and biophysical techniques, such as nuclear magnetic resonance and hydrogen-deuterium exchange coupled with mass spectrometry, are providing complementary insights into ligand-dependent dynamic equilibrium between different functional states. Additional details revealed by high-resolution structures illustrate the receptors as allosteric machines that are controlled not only by ligands but also by ions, lipids, cholesterol, and water. This wealth of data is helping redefine our knowledge of how GPCRs recognize such a diverse array of ligands and how they transmit signals 30 angstroms across the cell membrane; it also is shedding light on a structural basis of GPCR allosteric modulation and biased signaling.", "author" : [{ "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Annual review of pharmacology and toxicology", "id" : "ITEM-2", "issued" : { "date-parts" : [["2013", "1"]] }, "page" : "531-56", "title" : "Structure-function of the G protein-coupled receptor superfamily.", "type" : "article-journal", "volume" : "53" }, "uris" : ["http://www.mendeley.com/documents/?uuid=16227897-5681-4eba-96d1-07acca5ca220"] }], "mendeley" : { "formattedCitation" : "(Katritch et al., 2013; Venkatakrishnan et al., 2013)", "plainTextFormattedCitation" : "(Katritch et al., 2013; Venkatakrishnan et al., 2013)", "previouslyFormattedCitation" : "(Katritch et al., 2013; Venkatakrishnan et al., 2013)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . However, despite this wealth of structural information, it is still not fully understood how ligand binding leads to activated

receptors, which are able to trigger nucleotide exchange in intracellular effector G-protein complexes.

One of the major unknowns is the role of the highly conserved hydrophilic water-filled channel observed in crystal structures of class A GPCRs, which extends along the receptor axis from the external ligand-binding region nearly all the way to the effector binding site. The channel is sealed toward the cytoplasm by a thin layer of hydrophobic residues in inactive state GPCRs (Fig 1 A, B). High resolution X-ray structures of the inactive conformation reveal a Na^+ ion near the floor of this pocket, coordinated by water and three or four conserved residues including an acidic aspartate that is fully conserved in all ligand-sensing class A GPCRs {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature12944", "ISSN" : "0028-0836", "PMID" : "24413399", "abstract" : "Opioids represent widely prescribed and abused medications, although their signal transduction mechanisms are not well understood. Here we present the 1.8 \u00c5 high-resolution crystal structure of the human \u03b4-opioid receptor (\u03b4-OR), revealing the presence and fundamental role of a sodium ion in mediating allosteric control of receptor functional selectivity and constitutive activity. The distinctive \u03b4-OR sodium ion site architecture is centrally located in a polar interaction network in the seven-transmembrane bundle core, with the sodium ion stabilizing a reduced agonist affinity state, and thereby modulating signal transduction. Site-directed mutagenesis and functional studies reveal that changing the allosteric sodium site residue Asn 131 to an alanine or a valine augments constitutive \u03b2-arrestin-mediated signalling. Asp95Ala, Asn310Ala and Asn314Ala mutations transform classical \u03b4-opioid antagonists such as naltrindole into potent \u03b2-arrestin-biased agonists. The data establish the molecular basis for allosteric sodium ion control in opioid signalling, revealing that sodium-coordinating residues act as 'efficacy switches' at a prototypic G-protein-coupled receptor.", "author" : [{ "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Giguere", "given" : "Patrick M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huang", "given" : "Xi-Ping", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

"dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7487", "issued" : { "date-parts" : [["2014", "2", "13"]] }, "page" : "191-196", "publisher" : "Nature Publishing Group", "title" : "Molecular control of \u03b4-opioid receptor signalling", "type" : "article-journal", "volume" : "506" }, "uris" : ["http://www.mendeley.com/documents/?uuid=317fa7b9-9020-4478-801c-26c164d4fc45"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/nature11701", "ISBN" : "1476-4687 (Electronic)\\r0028-0836 (Linking)", "ISSN" : "1476-4687", "PMID" : "23222541", "abstract" : "Protease-activated receptor 1 (PAR1) is the prototypical member of a family of G-protein-coupled receptors that mediate cellular responses to thrombin and related proteases. Thrombin irreversibly activates PAR1 by cleaving the amino-terminal exodomain of the receptor, which exposes a tethered peptide ligand that binds the heptahelical bundle of the receptor to affect G-protein activation. Here we report the 2.2 \u00c5 resolution crystal structure of human PAR1 bound to vorapaxar, a PAR1 antagonist. The structure reveals an unusual mode of drug binding that explains how a small molecule binds virtually irreversibly to inhibit receptor activation by the tethered ligand of PAR1. In contrast to deep, solvent-exposed binding pockets observed in other peptide-activated G-protein-coupled receptors, the vorapaxar-binding pocket is superficial but has little surface exposed to the aqueous solvent. Protease-activated receptors are important targets for drug development. The structure reported here will aid the development of improved PAR1 antagonists and the discovery of antagonists to other members of this receptor family.", "author" : [{ "dropping-particle" : "", "family" : "Zhang", "given" : "Cheng", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Srinivasan", "given" : "Yoga", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Arlow", "given" : "Daniel H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fung", "given" : "Juan Jose", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palmer", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zheng", "given" : "Yaowu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" :

"Green", "given" : "Hillary F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pandey", "given" : "Anjali", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shaw", "given" : "David E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Coughlin", "given" : "Shaun R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-2", "issue" : "7429", "issued" : { "date-parts" : [["2012"]] }, "page" : "387-92", "publisher" : "Nature Publishing Group", "title" : "High-resolution crystal structure of human protease-activated receptor 1.", "type" : "article-journal", "volume" : "492" }, "uris" : ["http://www.mendeley.com/documents/?uuid=8da5d442-7451-481c-98b2-8ce3ec428a04"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1371/journal.pone.0092727", "ISSN" : "1932-6203", "PMID" : "24663151", "abstract" : "The \u03b21-adrenoceptor (\u03b21AR) is a G protein-coupled receptor (GPCR) that is activated by the endogenous agonists adrenaline and noradrenaline. We have determined the structure of an ultra-thermostable \u03b21AR mutant bound to the weak partial agonist cyanopindolol to 2.1 \u00c5 resolution. High-quality crystals (100 \u03bcm plates) were grown in lipidic cubic phase without the assistance of a T4 lysozyme or BRIL fusion in cytoplasmic loop 3, which is commonly employed for GPCR crystallisation. An intramembrane Na+ ion was identified co-ordinated to Asp872.50, Ser1283.39 and 3 water molecules, which is part of a more extensive network of water molecules in a cavity formed between transmembrane helices 1, 2, 3, 6 and 7. Remarkably, this water network and Na+ ion is highly conserved between \u03b21AR and the adenosine A2A receptor (rmsd of 0.3 \u00c5), despite an overall rmsd of 2.4 \u00c5 for all C\u03b1 atoms and only 23% amino acid identity in the transmembrane regions. The affinity of agonist binding and nanobody Nb80 binding to \u03b21AR is unaffected by Na+ ions, but the stability of the receptor is decreased by 7.5\u00b0C in the absence of Na+. Mutation of amino acid side chains that are involved in the co-ordination of either Na+ or water molecules in the network decreases the stability of \u03b21AR by 5-10\u00b0C. The data suggest that the intramembrane Na+ and associated water network stabilise the ligand-free state of \u03b21AR, but still permits the receptor to

form the activated state which involves the collapse of the Na+ binding pocket on agonist binding.", "author" : [{ "dropping-particle" : "", "family" : "Miller-Gallacher", "given" : "Jennifer L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nehm\u00e9", "given" : "Rony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Warne", "given" : "Tony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edwards", "given" : "Patricia C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schertler", "given" : "Gebhard F X", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Leslie", "given" : "Andrew G W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tate", "given" : "Christopher G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "PloS one", "id" : "ITEM-3", "issue" : "3", "issued" : { "date-parts" : [["2014", "1"]] }, "page" : "e92727", "title" : "The 2.1 \u00c5 Resolution Structure of Cyanopindolol-Bound \u03b21-Adrenoceptor Identifies an Intramembrane Na⁺ Ion that Stabilises the Ligand-Free Receptor.", "type" : "article-journal", "volume" : "9" }, "uris" : ["http://www.mendeley.com/documents/?uuid=af5847ea-2e45-4ed3-a743-fdff299ddb83"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1021/jm400140q", "ISBN" : "0022-2623", "ISSN" : "0022-2623", "PMID" : "23517028", "abstract" : "Biophysical fragment screening of a thermostabilized \u03b21-adrenergic receptor (\u03b21AR) using surface plasmon resonance (SPR) enabled the identification of moderate affinity, high ligand efficiency (LE) arylpiperazine hits 7 and 8. Subsequent hit to lead follow-up confirmed the activity of the chemotype, and a structure-based design approach using protein-ligand crystal structures of the \u03b21AR resulted in the identification of several fragments that bound with higher affinity, including indole 19 and quinoline 20. In the first example of GPCR crystallography with ligands derived from fragment screening, structures of the stabilized \u03b21AR complexed with 19 and 20 were determined at resolutions of 2.8 and 2.7 \u00c5, respectively.", "author" : [{ "dropping-particle" : "", "family" : "Christopher", "given" : "John A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Brown", "given" : "Jason", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dor\u00e9", "given" : "Andrew S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Errey", "given" : "James C.", "non-dropping-particle" :

"", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Koglin", "given" : "Markus", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Marshall", "given" : "Fiona H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Myszka", "given" : "David G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rich", "given" : "Rebecca L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tate", "given" : "Christopher G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tehan", "given" : "Benjamin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Warne", "given" : "Tony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Congreve", "given" : "Miles", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Medicinal Chemistry", "id" : "ITEM-4", "issue" : "9", "issued" : { "date-parts" : [["2013", "5", "9"]] }, "page" : "3446-3455", "title" : "Biophysical Fragment Screening of the \u03b2 1 -Adrenergic Receptor: Identification of High Affinity Arylpiperazine Leads Using Structure-Based Drug Design", "type" : "article-journal", "volume" : "56" }, "uris" : ["http://www.mendeley.com/documents/?uuid=22d16d02-9fa9-4753-8daf-f16f06d64a93"] }, { "id" : "ITEM-5", "itemData" : { "DOI" : "10.1038/nature10867", "ISBN" : "1476-4687 (Electronic)\\r0028-0836 (Linking)", "ISSN" : "0028-0836", "PMID" : "22358844", "abstract" : "Acetylcholine, the first neurotransmitter to be identified, exerts many of its physiological actions via activation of a family of G-protein-coupled receptors (GPCRs) known as muscarinic acetylcholine receptors (mAChRs). Although the five mAChR subtypes (M1-M5) share a high degree of sequence homology, they show pronounced differences in G-protein coupling preference and the physiological responses they mediate. Unfortunately, despite decades of effort, no therapeutic agents endowed with clear mAChR subtype selectivity have been developed to exploit these differences. We describe here the structure of the G(q/11)-coupled M3 mAChR ('M3 receptor', from rat) bound to the bronchodilator drug tiotropium and identify the binding mode for this clinically important drug. This structure, together with that of the G(i/o)-coupled M2 receptor, offers possibilities for the design of mAChR subtype-selective ligands. Importantly, the M3 receptor structure allows a structural comparison between two members of a mammalian GPCR subfamily displaying different G-protein coupling selectivities. Furthermore, molecular dynamics simulations suggest that tiotropium binds

transiently to an allosteric site en route to the binding pocket of both receptors. These simulations offer a structural view of an allosteric binding mode for an orthosteric GPCR ligand and provide additional opportunities for the design of ligands with different affinities or binding kinetics for different mAChR subtypes. Our findings not only offer insights into the structure and function of one of the most important GPCR families, but may also facilitate the design of improved therapeutics targeting these critical receptors.", "author" : [{ "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Jianxin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pan", "given" : "Albert C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Arlow", "given" : "Daniel H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosenbaum", "given" : "Daniel M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosemond", "given" : "Erica", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Green", "given" : "Hillary F.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Liu", "given" : "Tong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chae", "given" : "Pil Seok", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shaw", "given" : "David E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wess", "given" : "J\u00fcrgen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-5", "issued" : { "date-parts" : [["2012"]] }, "page" : "552-556", "title" : "Structure and dynamics of the M3 muscarinic acetylcholine receptor", "type" : "article", "volume" : "482" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ded92c1a-a8e9-4a39-be6f-63095da52e45"] }, { "id" : "ITEM-6", "itemData" : { "DOI" : "10.1126/science.1219218", "ISBN" : "0036-8075", "ISSN" : "1095-9203", "PMID" : "22798613", "abstract" : "Pharmacological responses of G protein-coupled receptors

(GPCRs) can be fine-tuned by allosteric modulators. Structural studies of such effects have been limited due to the medium resolution of GPCR structures. We reengineered the human A(2A) adenosine receptor by replacing its third intracellular loop with apocytochrome b(562)RIL and solved the structure at 1.8 angstrom resolution. The high-resolution structure allowed us to identify 57 ordered water molecules inside the receptor comprising three major clusters. The central cluster harbors a putative sodium ion bound to the highly conserved aspartate residue Asp(2.50). Additionally, two cholesterols stabilize the conformation of helix VI, and one of 23 ordered lipids intercalates inside the ligand-binding pocket. These high-resolution details shed light on the potential role of structured water molecules, sodium ions, and lipids/cholesterol in GPCR stabilization and function.",
"author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Wei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chun", "given" : "Eugene", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chubukov", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xu", "given" : "Fei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Gye Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Christopher B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "Adriaan P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science (New York, N.Y.)", "id" : "ITEM-6", "issue" : "6091", "issued" : { "date-parts" : [["2012", "7", "13"]] }, "page" : "232-6", "title" : "Structural basis for allosteric regulation of GPCRs by sodium ions.", "type" : "article-journal", "volume" : "337" }, "uris" : ["http://www.mendeley.com/documents/?uuid=81df11c9-4f2a-4a4f-8c01-0de1af0d5e09"] }, { "id" : "ITEM-7", "itemData" : { "DOI" : "10.1002/cbic.200600429", "ISBN" : "1439-

7633", "ISSN" : "14394227", "PMID" : "17173267", "abstract" : "Not so watered down. In this review, we summarize the role of water molecules embedded in the transmembrane bundle of G protein-coupled receptors in stabilizing intra- and interhelical interactions, and their use for building computer-generated homology models. \u00a9 2007 Wiley-VCH Verlag GmbH & Co. KGaA.", "author" : [{ "dropping-particle" : "", "family" : "Pardo", "given" : "Leonardo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Deupi", "given" : "Xavier", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "D\u00f6lker", "given" : "Nicole", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "L\u00f3pez-Rodr\u00edguez", "given" : "Mar\u00eda Luz", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Campillo", "given" : "Mercedes", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "ChemBioChem", "id" : "ITEM-7", "issue" : "1", "issued" : { "date-parts" : [["2007"]] }, "page" : "19-24", "title" : "The role of internal water molecules in the structure and function of the rhodopsin family of G protein-coupled receptors", "type" : "article", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f7549695-773e-45cc-8444-26d05e71ede7"] }], "mendeley" : { "formattedCitation" : "(Christopher et al., 2013; Fenalti et al., 2014; Kruse et al., 2012; Liu et al., 2012; Miller-Gallacher et al., 2014; Pardo et al., 2007; Zhang et al., 2012)", "plainTextFormattedCitation" : "(Christopher et al., 2013; Fenalti et al., 2014; Kruse et al., 2012; Liu et al., 2012; Miller-Gallacher et al., 2014; Pardo et al., 2007; Zhang et al., 2012)", "previouslyFormattedCitation" : "(Christopher et al., 2013; Fenalti et al., 2014; Kruse et al., 2012; Liu et al., 2012; Miller-Gallacher et al., 2014; Pardo et al., 2007; Zhang et al., 2012)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** ($D^{2.50}$; superscript refers to the Ballesteros and Weinstein residue numbering system) **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.tips.2014.11.001", "ISBN" : "1873-3735 (Electronic)\\r0165-6147 (Linking)", "ISSN" : "01656147", "PMID" : "25541108", "abstract" : "Generic residue numbers facilitate comparisons of, for example, mutational effects, ligand interactions, and structural motifs. The numbering scheme by Ballesteros and Weinstein for residues within the class A GPCRs (G protein-coupled receptors) has more than 1100 citations, and the recent crystal structures for classes B, C, and F now call for a community consensus in residue numbering within and

across these classes. Furthermore, the structural era has uncovered helix bulges and constrictions that offset the generic residue numbers. The use of generic residue numbers depends on convenient access by pharmacologists, chemists, and structural biologists. We review the generic residue numbering schemes for each GPCR class, as well as a complementary structure-based scheme, and provide illustrative examples and GPCR database (GPCRDB) web tools to number any receptor sequence or structure.", "author" : [{ "dropping-particle" : "", "family" : "Isberg", "given" : "Vignir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Graaf", "given" : "Chris", "non-dropping-particle" : "de", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bortolato", "given" : "Andrea", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Marshall", "given" : "Fiona H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mordalski", "given" : "Stefan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pin", "given" : "Jean-Philippe", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vriend", "given" : "Gerrit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gloriam", "given" : "David E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Pharmacological Sciences", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2015", "1"]] }, "page" : "22-31", "publisher" : "Elsevier Ltd", "title" : "Generic GPCR residue numbers \u2013 aligning topology maps while minding the gaps", "type" : "article-journal", "volume" : "36" }, "uris" : ["http://www.mendeley.com/documents/?uuid=069e307d-2306-4fdf-af5b-404cb9d34de4"] }], "mendeley" : { "formattedCitation" : "(Isberg et al., 2015)", "plainTextFormattedCitation" : "(Isberg et al., 2015)", "previouslyFormattedCitation" : "(Isberg et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . The allosteric effect of monovalent cations, in particular Na^+ ions, for GPCR function has been known for almost half a century **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" :

"ITEM-1", "itemData" : { "abstract" : "Receptor binding of the tritiated opiate antagonists naloxone, nalorphine, and levallorphan is enhanced by sodium ion, while binding of the tritiated agonists oxymorphone, dihydromorphine, and levorphanol is diminished. This differential effect of Na+ is highly specific, since it is elicited by Na+ and Li+ but not by other monovalent or divalent cations. The relative effectiveness of nonradioactive opiates in inhibiting [3H]naloxone binding, in the absence and presence of Na+ in vitro, correlates well with their relative agonist antagonist properties in vivo. It is hypothesized that sodium allosterically transforms opiate receptor sites from conformations which bind agonists more readily, to conformations which bind antagonists more readily. This hypothesis is supported by the competition of opiate agonists and antagonists for receptor sites, the marked temperature dependence of binding, the similar extent of binding of tritiated agonists and antagonists at maximal saturation, the concurrent increase in naloxone binding sites and decrease in dihydromorphine binding sites caused by the addition of Na+, and the ability of Na+ to increase [3H] dihydromorphine dissociation, with no effect on [3H]naloxone dissociation.", "author" : [{ "dropping-particle" : "", "family" : "Pert", "given" : "Candace B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Synder", "given" : "Solomon H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular pharmacology", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["1974", "4", "1"]] }, "page" : "868-879", "title" : "Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium", "type" : "article-journal", "volume" : "10" }, "uris" : ["http://www.mendeley.com/documents/?uuid=36170cac-168d-403c-9106-8c9832a9dbd9"] }], "mendeley" : { "formattedCitation" : "(Pert and Synder, 1974)", "plainTextFormattedCitation" : "(Pert and Synder, 1974)", "previouslyFormattedCitation" : "(Pert and Synder, 1974)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** , and the bulk of recent evidence shows that these effects are largely mediated by the ion binding at the $D^{2.50}$ site at the physiological concentration of Na^{+} (140 mM and lower) **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1126/science.1219218", "ISBN" : "0036-8075", "ISSN" : "1095-9203", "PMID" : "22798613", "abstract" : "Pharmacological responses of G protein-coupled receptors (GPCRs) can be fine-tuned by allosteric modulators. Structural studies of such effects have been limited due to the medium resolution of GPCR structures. We reengineered the human A(2A) adenosine receptor by replacing its third intracellular loop with

apocytochrome b(562)RIL and solved the structure at 1.8 angstrom resolution. The high-resolution structure allowed us to identify 57 ordered water molecules inside the receptor comprising three major clusters. The central cluster harbors a putative sodium ion bound to the highly conserved aspartate residue Asp(2.50). Additionally, two cholesterols stabilize the conformation of helix VI, and one of 23 ordered lipids intercalates inside the ligand-binding pocket. These high-resolution details shed light on the potential role of structured water molecules, sodium ions, and lipids/cholesterol in GPCR stabilization and function.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Wei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chun", "given" : "Eugene", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chubukov", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xu", "given" : "Fei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Gye Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Christopher B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "Adriaan P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science (New York, N.Y.)", "id" : "ITEM-1", "issue" : "6091", "issued" : { "date-parts" : [["2012", "7", "13"]] }, "page" : "232-6", "title" : "Structural basis for allosteric regulation of GPCRs by sodium ions.", "type" : "article-journal", "volume" : "337" }, "uris" : ["http://www.mendeley.com/documents/?uuid=81df11c9-4f2a-4a4f-8c01-0de1af0d5e09"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1124/mol.114.095737", "ISSN" : "1521-0111", "author" : [{ "dropping-particle" : "", "family" : "Massink", "given" : "Arnault", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gutierrez-de-Teran", "given" : "H.", "non-dropping-particle" : "", "parse-

names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lenselink", "given" : "Eelke B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "V.", "family" : "Ortiz Zacarias", "given" : "N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xia", "given" : "Lizi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "A. P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular Pharmacology", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2015", "1", "7"]] }, "page" : "305-313", "title" : "Sodium Ion Binding Pocket Mutations and Adenosine A2A Receptor Function", "type" : "article-journal", "volume" : "87" }, "uris" : ["http://www.mendeley.com/documents/?uuid=bf1014bc-5386-4fde-acd3-eea4821cd7de"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1038/nature12944", "ISSN" : "0028-0836", "PMID" : "24413399", "abstract" : "Opioids represent widely prescribed and abused medications, although their signal transduction mechanisms are not well understood. Here we present the 1.8 \u00c5 high-resolution crystal structure of the human \u03b4-opioid receptor (\u03b4-OR), revealing the presence and fundamental role of a sodium ion in mediating allosteric control of receptor functional selectivity and constitutive activity. The distinctive \u03b4-OR sodium ion site architecture is centrally located in a polar interaction network in the seven-transmembrane bundle core, with the sodium ion stabilizing a reduced agonist affinity state, and thereby modulating signal transduction. Site-directed mutagenesis and functional studies reveal that changing the allosteric sodium site residue Asn 131 to an alanine or a valine augments constitutive \u03b2-arrestin-mediated signalling. Asp95Ala, Asn310Ala and Asn314Ala mutations transform classical \u03b4-opioid antagonists such as naltrindole into potent \u03b2-arrestin-biased agonists. The data establish the molecular basis for allosteric sodium ion control in opioid signalling, revealing that sodium-coordinating residues act as 'efficacy switches' at a prototypic G-protein-coupled receptor.", "author" : [{ "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Giguere", "given" : "Patrick M",

"non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huang", "given" : "Xi-Ping", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-3", "issue" : "7487", "issued" : { "date-parts" : [["2014", "2", "13"]] }, "page" : "191-196", "publisher" : "Nature Publishing Group", "title" : "Molecular control of \u03b4-opioid receptor signalling", "type" : "article-journal", "volume" : "506" }, "uris" : ["http://www.mendeley.com/documents/?uuid=317fa7b9-9020-4478-801c-26c164d4fc45"] }], "mendeley" : { "formattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)", "plainTextFormattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)", "previouslyFormattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Due to the highly conserved nature of $D^{2.50}$ and other Na^+ ion coordinating residues, Na^+ ion binding at this site is likely to be a ubiquitous feature shared by the vast majority of class A GPCRs **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.tibs.2014.03.002", "ISBN" : "0968-0004", "ISSN" : "09680004", "PMID" : "24767681", "abstract" : "Despite their functional and structural diversity, G-protein-coupled receptors (GPCRs) share a common mechanism of signal transduction via conformational changes in the seven-transmembrane (7TM) helical domain. New major insights into this mechanism come from the recent crystallographic discoveries of a partially hydrated sodium ion that is specifically bound in the middle of the 7TM bundle of multiple class A GPCRs. This review discusses the remarkable structural conservation and distinct features of the Na+ pocket in this most populous GPCR class, as well as the conformational collapse of the pocket upon receptor activation. New insights help to explain allosteric effects of sodium on GPCR agonist binding and activation, and sodium's role as a potential co-factor in class A GPCR function. \u00a9 2014 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" :

"Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Abola", "given" : "Enrique E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Biochemical Sciences", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["2014", "5"]] }, "page" : "233-244", "publisher" : "Elsevier Ltd", "title" : "Allosteric sodium in class A GPCR signaling", "type" : "article", "volume" : "39" }, "uris" : ["http://www.mendeley.com/documents/?uuid=813b6a82-51e4-47f8-9191-e3f93ac1b280"] }], "mendeley" : { "formattedCitation" : "(Katritch et al., 2014)", "plainTextFormattedCitation" : "(Katritch et al., 2014)", "previouslyFormattedCitation" : "(Katritch et al., 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

In active receptor conformations, the ion binding site near $D^{2.50}$ shows a collapsed state, which is likely not optimal for Na^+ ion binding **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1126/science.1219218", "ISBN" : "0036-8075", "ISSN" : "1095-9203", "PMID" : "22798613", "abstract" : "Pharmacological responses of G protein-coupled receptors (GPCRs) can be fine-tuned by allosteric modulators. Structural studies of such effects have been limited due to the medium resolution of GPCR structures. We reengineered the human A(2A) adenosine receptor by replacing its third intracellular loop with apocytochrome b(562)RIL and solved the structure at 1.8 angstrom resolution. The high-resolution structure allowed us to identify 57 ordered water molecules inside the receptor comprising three major clusters. The central cluster harbors a putative sodium ion bound to the highly conserved aspartate residue Asp(2.50). Additionally, two cholesterols stabilize the conformation of helix VI, and one of 23 ordered lipids intercalates inside the ligand-binding pocket. These high-resolution details shed light on the potential role of structured water molecules, sodium ions, and lipids/cholesterol in GPCR stabilization and function.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Wei", "non-dropping-particle" : "", "parse-names" : false,

"suffix" : "" }, { "dropping-particle" : "", "family" : "Chun", "given" : "Eugene", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chubukov", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xu", "given" : "Fei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Gye Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Christopher B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "Adriaan P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science (New York, N.Y.)", "id" : "ITEM-1", "issue" : "6091", "issued" : { "date-parts" : [["2012", "7", "13"]] }, "page" : "232-6", "title" : "Structural basis for allosteric regulation of GPCRs by sodium ions.", "type" : "article-journal", "volume" : "337" }, "uris" : ["http://www.mendeley.com/documents/?uuid=81df11c9-4f2a-4a4f-8c01-0de1af0d5e09"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/nature10361", "ISSN" : "1476-4687", "PMID" : "21772288", "abstract" : "G protein-coupled receptors (GPCRs) are responsible for the majority of cellular responses to hormones and neurotransmitters as well as the senses of sight, olfaction and taste. The paradigm of GPCR signalling is the activation of a heterotrimeric GTP binding protein (G protein) by an agonist-occupied receptor. The \u03b2(2) adrenergic receptor (\u03b2(2)AR) activation of Gs, the stimulatory G protein for adenylyl cyclase, has long been a model system for GPCR signalling. Here we present the crystal structure of the active state ternary complex composed of agonist-occupied monomeric \u03b2(2)AR and nucleotide-free Gs heterotrimer. The principal interactions between the \u03b2(2)AR and Gs involve the amino- and carboxy-terminal \u03b1-helices of Gs, with conformational changes propagating to the nucleotide-binding pocket. The largest conformational changes in the \u03b2(2)AR include a 14 \u00c5 outward movement at the cytoplasmic end of

transmembrane segment 6 (TM6) and an \u03b1-helical extension of the cytoplasmic end of TM5. The most surprising observation is a major displacement of the \u03b1-helical domain of G\u03b1s relative to the Ras-like GTPase domain. This crystal structure represents the first high-resolution view of transmembrane signalling by a GPCR.", "author" : [{ "dropping-particle" : "", "family" : "Rasmussen", "given" : "S\u00f8ren G F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "DeVree", "given" : "Brian T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zou", "given" : "Yaozhong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chung", "given" : "Ka Young", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Tong Sun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thian", "given" : "Foon Sun", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chae", "given" : "Pil Seok", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pardon", "given" : "Els", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Calinski", "given" : "Diane", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mathiesen", "given" : "Jesper M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shah", "given" : "Syed T a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lyons", "given" : "Joseph a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Caffrey", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gellman", "given" : "Samuel H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Skiniotis", "given" : "Georgios", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle"

: "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-2", "issue" : "7366", "issued" : { "date-parts" : [["2011", "9", "29"]] }, "page" : "549-55", "publisher" : "Nature Publishing Group", "title" : "Crystal structure of the \u03b22 adrenergic receptor-Gs protein complex.", "type" : "article-journal", "volume" : "477" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a516d094-a5f0-40ea-abfd-38991b44ff9c"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1038/nature12735", "ISSN" : "0028-0836", "PMID" : "24256733", "abstract" : "Despite recent advances in crystallography and the availability of G-protein-coupled receptor (GPCR) structures, little is known about the mechanism of their activation process, as only the \u03b22 adrenergic receptor (\u03b22AR) and rhodopsin have been crystallized in fully active conformations. Here we report the structure of an agonist-bound, active state of the human M2 muscarinic acetylcholine receptor stabilized by a G-protein mimetic camelid antibody fragment isolated by conformational selection using yeast surface display. In addition to the expected changes in the intracellular surface, the structure reveals larger conformational changes in the extracellular region and orthosteric binding site than observed in the active states of the \u03b22AR and rhodopsin. We also report the structure of the M2 receptor simultaneously bound to the orthosteric agonist iperoxo and the positive allosteric modulator LY2119620. This structure reveals that LY2119620 recognizes a largely pre-formed binding site in the extracellular vestibule of the iperoxo-bound receptor, inducing a slight contraction of this outer binding pocket. These structures offer important insights into the activation mechanism and allosteric modulation of muscarinic receptors.", "author" : [{ "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ring", "given" : "Aaron M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Jianxin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Kelly", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Eitel", "given" : "Katrin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "H\u00fcbner", "given" : "Harald", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pardon", "given" : "Els", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Valant", "given" : "Celine", "non-dropping-particle" :

"", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sexton", "given" : "Patrick M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Christopoulos", "given" : "Arthur", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Felder", "given" : "Christian C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gmeiner", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Garcia", "given" : "K Christopher", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wess", "given" : "J\u00fcrgen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-3", "issue" : "7478", "issued" : { "date-parts" : [["2013", "11", "20"]] }, "page" : "101-106", "title" : "Activation and allosteric modulation of a muscarinic acetylcholine receptor", "type" : "article-journal", "volume" : "504" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ad341a17-7bbd-47c2-8fdc-51826b530f13"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1038/nature14886", "ISSN" : "0028-0836", "author" : [{ "dropping-particle" : "", "family" : "Huang", "given" : "Weijiao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Venkatakrishnan", "given" : "A J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Laeremans", "given" : "Toon", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Feinberg", "given" : "Evan N", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sanborn", "given" : "Adrian L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kato", "given" : "Hideaki E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Livingston", "given" : "Kathryn E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thorsen", "given" : "Thor S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-

particle" : "", "family" : "Kling", "given" : "Ralf C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Granier", "given" : "S\u00e9bastien", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gmeiner", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Husbands", "given" : "Stephen M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Traynor", "given" : "John R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-4", "issue" : "7565", "issued" : { "date-parts" : [["2015", "8", "5"]] }, "page" : "315-321", "title" : "Structural insights into \u00b5-opioid receptor activation", "type" : "article-journal", "volume" : "524" }, "uris" : ["http://www.mendeley.com/documents/?uuid=25d8632d-4fd4-478d-b0ef-03c07f2f5348"] }], "mendeley" : { "formattedCitation" : "(Huang et al., 2015; Kruse et al., 2013; Liu et al., 2012; Rasmussen et al., 2011)", "plainTextFormattedCitation" : "(Huang et al., 2015; Kruse et al., 2013; Liu et al., 2012; Rasmussen et al., 2011)", "previouslyFormattedCitation" : "(Huang et al., 2015; Kruse et al., 2013; Liu et al., 2012; Rasmussen et al., 2011)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. It was therefore proposed that the Na^+ ion leaves the hydrophilic pocket upon receptor activation by a ligand or during receptor-G-protein complex formation. However, how this movement is triggered and which pathway is followed by the ion remains unknown.

Here, we investigated the link between ligand-induced receptor activation, the fate of the bound Na^+ ion in class A GPCRs and its implications for transmembrane (TM) signal transduction by equilibrium and non-equilibrium atomistic simulations on the M2 muscarinic receptor (m2r). When one addresses these questions, it is important to take physiologically relevant electrochemical membrane conditions into consideration. Strong TM Na^+ and K^+ gradients produce a sizable voltage across the plasma membrane of up

to -100 mV in the resting state of mammalian cells {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1036/0838577016", "ISBN" : "0838577016", "abstract" : "Important features to this edition include a new chapter - Genes and Behavior; a complete updating of development of the nervous system; the genetic basis of...", "author" : [{ "dropping-particle" : "", "family" : "Kandel", "given" : "E R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schwartz", "given" : "J H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jessell", "given" : "T M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Mcgraw-Hill Publ.Comp.", "id" : "ITEM-1", "issued" : { "date-parts" : [["2000"]] }, "number-of-pages" : "1414", "title" : "Principles of Neural Science", "type" : "book", "volume" : "3" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f222e128-e0e6-40ee-9fa7-528b1c91a7d0"] }], "mendeley" : { "formattedCitation" : "(Kandel et al., 2000)", "plainTextFormattedCitation" : "(Kandel et al., 2000)", "previouslyFormattedCitation" : "(Kandel et al., 2000)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }}. Both the ionic gradients and electric field have been shown to influence the function of GPCRs {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1113/jphysiol.2010.204107", "ISSN" : "1469-7793", "PMID" : "21282291", "abstract" : "The ability to sense transmembrane voltage is a central feature of many membrane proteins, most notably voltage-gated ion channels. Gating current measurements provide valuable information on protein conformational changes induced by voltage. The recent observation that muscarinic G-protein-coupled receptors (GPCRs) generate gating currents confirms their intrinsic capacity to sense the membrane electrical field. Here, we studied the effect of voltage on agonist activation of M2 muscarinic receptors (M2R) in atrial myocytes and how agonist binding alters M2R gating currents. Membrane depolarization decreased the potency of acetylcholine (ACh), but increased the potency and efficacy of pilocarpine (Pilo), as measured by ACh-activated K+ current, I(KACh). Voltage-induced conformational changes in M2R were modified in a ligand-selective manner: ACh reduced gating charge displacement while Pilo increased the amount of charge displaced. Thus, these ligands manifest opposite voltage-dependent I(KACh) modulation and exert opposite effects on M2R gating charge displacement. Finally, mutations in the putative ligand binding site perturbed the movement of the M2R voltage sensor. Our data suggest that changes in voltage induce conformational changes in

the ligand binding site that alter the agonist\u2013receptor interaction in a ligand-dependent manner. Voltage-dependent GPCR modulation has important implications for cellular signalling in excitable tissues. Gating current measurement allows for the tracking of subtle conformational changes in the receptor that accompany agonist binding and changes in membrane voltage.", "author" : [{ "dropping-particle" : "", "family" : "Navarro-Polanco", "given" : "Ricardo a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Moreno Galindo", "given" : "Eloy G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ferrer-Villada", "given" : "Tania", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Arias", "given" : "Marcelo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rigby", "given" : "J Ryan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "S\u00e1nchez-Chapula", "given" : "Jos\u00e9 a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tristani-Firouzi", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of physiology", "id" : "ITEM-1", "issue" : "Pt 7", "issued" : { "date-parts" : [["2011"]] }, "page" : "1741-1753", "title" : "Conformational changes in the M2 muscarinic receptor induced by membrane voltage and agonist binding.", "type" : "article-journal", "volume" : "589" }, "uris" : ["http://www.mendeley.com/documents/?uuid=d63d2c04-3f65-43b4-a700-55a904c9a3bd"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/nature05259", "ISSN" : "1476-4687", "PMID" : "17065983", "abstract" : "Activation by agonist binding of G-protein-coupled receptors (GPCRs) controls most signal transduction processes. Although these receptors span the cell membrane, they are not considered to be voltage sensitive. Recently it was shown that both the activity of GPCRs and their affinity towards agonists are regulated by membrane potential. However, it remains unclear whether GPCRs intrinsically respond to changes in membrane potential. Here we show that two prototypical GPCRs, the m2 and m1 muscarinic receptors (m2R and m1R), display charge-movement-associated currents analogous to 'gating currents' of voltage-gated channels. The gating charge-voltage relationship of m2R correlates well with the voltage dependence of the affinity of the receptor for acetylcholine. The loop that couples m2R and m1R to their G protein has a crucial function in coupling voltage sensing to agonist-binding affinity. Our data strongly indicate that GPCRs serve as sensors for both transmembrane potential and external chemical signals.", "author" : [{ "dropping-particle"

: "", "family" : "Ben-Chaim", "given" : "Yair", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chanda", "given" : "Baron", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dascal", "given" : "Nathan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bezanilla", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parnas", "given" : "Itzchak", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parnas", "given" : "Hanna", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-2", "issue" : "7115", "issued" : { "date-parts" : [["2006", "11", "2"]] }, "page" : "106-9", "title" : "Movement of 'gating charge' is coupled to ligand binding in a G-protein-coupled receptor.", "type" : "article-journal", "volume" : "444" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c37d2319-476d-4482-bc57-24d9e0725fc5"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1126/scisignal.aac7419", "ISSN" : "1945-0877", "author" : [{ "dropping-particle" : "", "family" : "Rinne", "given" : "Andreas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mobarec", "given" : "Juan Carlos", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahaut-Smith", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kolb", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bunemann", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science Signaling", "id" : "ITEM-3", "issue" : "401", "issued" : { "date-parts" : [["2015", "11", "3"]] }, "page" : "ra110-ra110", "title" : "The mode of agonist binding to a G protein-coupled receptor switches the effect that voltage changes have on signaling", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f3420ad6-cf1a-485e-8bf7-120a7866c9ae"] }], "mendeley" : { "formattedCitation" : "(Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011; Rinne et al., 2015)", "plainTextFormattedCitation" : "(Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011; Rinne et al., 2015)", "previouslyFormattedCitation" : "(Ben-Chaim et al., 2006; Navarro-Polanco et al., 2011; Rinne et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** and are likely to impact the movement of the Na^+ ion within the membrane region.

Our data reveal that the Na^+ ion observed in the TM domain of class A GPCRs can readily traverse the receptor and, driven by the electrochemical gradients, migrate into the cytoplasm in active receptor conformations. This result implies that a Na^+ ion may be exchanged from the extracellular space to the cytoplasm as an important step in receptor activation. Furthermore, the movement of Na^+ in the receptor, and intracellular egress, are coupled to a protonation change of $D^{2.50}$.

RESULTS

GPCR activation opens a hydrated pathway across the receptor

We were first interested whether the conformational change from the inactive to active receptor state renders the ion-binding pocket sterically incapable of accommodating a Na^+ ion. The binding site for Na^+ appears to adopt a collapsed conformation in active crystal structures. We started from an inactive state structure of the m2 muscarinic acetylcholine receptor (m2r, PDB ID: 3UON) and, using a targeted molecular dynamics (TMD) approach, gently drove this conformation to the active state of this receptor (PDB ID: 4MQT) (Fig S1).

Our simulations show that the active state of m2r initially retains sufficient space for the ion. The electrostatic attraction between the ion and the negatively charged side chain of $D69^{2.50}$ keeps the ion bound to this site during and after the transition from the inactive to the active receptor conformation (Fig S1). However, our simulations show a widening of the intracellular portion of the TM helices below the hydrophilic pocket during this conformational change, which subsequently becomes fully hydrated (Fig 1 B, C). The hydrated pathway forms a connection between the orthosteric ligand-binding site, the hydrophilic pocket and the G-protein binding site. The slim hydrophobic layer that delimits the hydrophilic pocket toward the G-protein binding site in the inactive crystal structure undergoes substantial conformational changes, which are especially evident from the sidechain position of $Y440^{7.53}$. Our simulations show two major conformations of the $Y440^{7.53}$ sidechain following the transition – an upward state similar to the conformation observed in the inactive crystal structure (PDB: 3UON; Fig 1 D) and a downward configuration, which is also seen in the active crystal structure (PDB: 4MQT; Fig 1 E). The formation of a hydrated pathway connecting the receptor ligand and effector binding sites has been reported in previous simulation studies on the $A_{2A}R$ and 5-HT_{1A} receptors {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/ncomms5733", "ISSN" : "2041-1723", "author" : [{ "dropping-particle" : "", "family" : "Yuan", "given" : "Shuguang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filipek", "given" : "Slawomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palczewski", "given" : "Krzysztof", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vogel", "given" : "Horst", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature Communications", "id" : "ITEM-1", "issue" : "May", "issued" : { "date-parts" : [["2014"]]

}, "page" : "4733", "publisher" : "Nature Publishing Group", "title" : "Activation of G-protein-coupled receptors correlates with the formation of a continuous internal water pathway", "type" : "article-journal", "volume" : "5" }, "uris" : ["http://www.mendeley.com/documents/?uuid=fb3a2654-15f5-430a-947a-d743d513e2bb"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1002/anie.201603766", "ISSN" : "14337851", "author" : [{ "dropping-particle" : "", "family" : "Yuan", "given" : "Shuguang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Peng", "given" : "Qian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palczewski", "given" : "Krzysztof", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vogel", "given" : "Horst", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filipek", "given" : "Slawomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Angewandte Chemie International Edition", "id" : "ITEM-2", "issue" : "30", "issued" : { "date-parts" : [["2016", "7", "18"]] }, "page" : "8661-8665", "title" : "Mechanistic Studies on the Stereoselectivity of the Serotonin 5-HT 1A Receptor", "type" : "article-journal", "volume" : "55" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9e6beb21-d6a0-46a9-ae07-ae8726b9470d"] }], "mendeley" : { "formattedCitation" : "(Yuan et al., 2014, 2016)", "plainTextFormattedCitation" : "(Yuan et al., 2014, 2016)", "previouslyFormattedCitation" : "(Yuan et al., 2014, 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** , however the previous reports did not take the presence of a Na^+ ion into consideration.

The position of the internal Na^+ ion is coupled to protonation of D2.50

We were next interested in the interplay between the Na^+ ion and the key conserved titratable residue $D69^{2.50}$. A number of computational studies have explored functional implications of the protonation state of $D^{2.50}$, in particular its role in receptor activation, Na^+ ion binding, and interaction with the "ionic lock" motif ($DR^{3.50}Y$) in several class A family GPCRs **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1021/bi5008723", "ISSN" : "0006-2960", "author" : [{ "dropping-particle" : "", "family" : "Ranganathan", "given" : "Anirudh", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Carlsson", "given" : "Jens", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biochemistry", "id" : "ITEM-1", "issue" : "46", "issued" : { "date-parts" : [["2014"]] }, "page" : "7283-7296", "title" : "Insights into the Role of Asp79^{2.50} in \u03b22 Adrenergic Receptor Activation from Molecular Dynamics Simulations", "type" : "article-journal", "volume" : "53" }, "uris" : ["http://www.mendeley.com/documents/?uuid=8f2b05de-fb6d-493f-9bce-9cd4277ff6b0"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.bpj.2015.03.003", "ISSN" : "00063495", "author" : [{ "dropping-particle" : "", "family" : "Miao", "given" : "Yinglong", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Caliman", "given" : "Alisha D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "McCammon", "given" : "J. Andrew", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical Journal", "id" : "ITEM-2", "issue" : "7", "issued" : { "date-parts" : [["2015"]] }, "page" : "1796-1806", "publisher" : "Biophysical Society", "title" : "Allosteric Effects of Sodium Ion Binding on Activation of the M3 Muscarinic G-Protein-Coupled Receptor", "type" : "article-journal", "volume" : "108" }, "uris" : ["http://www.mendeley.com/documents/?uuid=88343e2a-b508-4813-9b0e-7fd42e8dd98d"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1016/j.jmb.2010.01.060", "ISBN" : "1089-8638 (Electronic)\\r0022-2836 (Linking)", "ISSN" : "00222836", "PMID" : "20132827", "abstract" : "The mechanism of signal transduction in G-protein-coupled receptors (GPCRs) is a crucial step in cell signaling. However, the molecular details of this process are still largely undetermined. Carrying out submicrosecond molecular dynamics simulations of ??-adrenergic receptors, we found that cooperation between a number of highly conserved residues is crucial to alter the equilibrium between the active state and the inactive state of

diffusible ligand GPCRs. In particular, \"ionic-lock\" formation in ??-adrenergic receptors is directly correlated with the protonation state of a highly conserved aspartic acid residue [Asp(2.50)] even though the two sites are located more than 20 ?? away from each other. Internal polar residues, acting as local microswitches, cooperate to propagate the signal from Asp(2.50) to the G-protein interaction site at the helix III-helix VI interface. Evolutionarily conserved differences between opsin and non-opsin GPCRs in the surrounding of Asp(2.50) influence the acidity of this residue and can thus help in rationalizing the differences in constitutive activity of class A GPCRs. ?? 2010 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Vanni", "given" : "Stefano", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Neri", "given" : "Marilisa", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tavernelli", "given" : "Ivano", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rothlisberger", "given" : "Ursula", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Molecular Biology", "id" : "ITEM-3", "issue" : "5", "issued" : { "date-parts" : [["2010", "4"]] }, "page" : "1339-1349", "publisher" : "Elsevier Ltd", "title" : "A Conserved Protonation-Induced Switch can Trigger \u201cIonic-Lock\u201d Formation in Adrenergic Receptors", "type" : "article-journal", "volume" : "397" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f22f1be0-ba40-4b9b-94ac-4de35e1f7feb"] }], "mendeley" : { "formattedCitation" : "(Miao et al., 2015; Ranganathan et al., 2014; Vanni et al., 2010)", "plainTextFormattedCitation" : "(Miao et al., 2015; Ranganathan et al., 2014; Vanni et al., 2010)", "previouslyFormattedCitation" : "(Miao et al., 2015; Ranganathan et al., 2014; Vanni et al., 2010)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}**. Here, we focused on a potential coupling between the position of the Na^+ ion within the receptor and protonation of $D69^{2.50}$. We carried out pK_a calculations on $D69^{2.50}$ using more than 800 equilibrated frames from simulations of the m2r receptor in a variety of conformations, including both the upward and downward configurations of the $Y440^{7.53}$ sidechain. Due to the formation of a hydrated pathway across the receptor from the ligand to the effector binding sites in the active state simulations, we were able to evaluate the effect of the Na^+ ion positional changes on the $D69^{2.50}$ pK_a, where the Na^+ ion was shifted both in the upward (toward the extracellular face) and downward direction.

Figure 2 shows that the pK_a value and, thus, the most likely protonation state of D69$^{2.50}$ are substantially influenced by the Na^+ ion. If the cation is within ~3-5 Å of D69$^{2.50}$, its positive charge strongly stabilizes the negatively charged form of D69$^{2.50}$, leading to a pK_a value of ~3–4. However, displacement of the Na^+ ion to distances of 5 Å and greater gives rise to a substantial pK_a shift to values between 8-12. This can be understood given the location of D69$^{2.50}$ in the middle of the transmembrane domain, surrounded by many non-polar residues. Transient movements of the internal Na^+ ion from its binding site, facilitated by activation-related conformational changes in the Na^+ pocket, can therefore be sufficient to lead to protonation of D69$^{2.50}$.

For the protonation of D$^{2.50}$, we propose that the most likely proton entry route would be from the extracellular side, along the negative membrane potential gradient. Moreover, in the m2r and other aminergic receptors the proton could be transferred from the conserved D$^{3.32}$ in the orthosteric binding pocket via a short chain of water molecules **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1073/pnas.1417888112", "ISSN" : "0027-8424", "author" : [{ "dropping-particle" : "", "family" : "Isom", "given" : "Daniel G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dohlman", "given" : "Henrik G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Proceedings of the National Academy of Sciences", "id" : "ITEM-1", "issued" : { "date-parts" : [["2015"]] }, "page" : "201417888", "title" : "Buried ionizable networks are an ancient hallmark of G protein-coupled receptor activation", "type" : "article-journal", "volume" : "2015" }, "uris" : ["http://www.mendeley.com/documents/?uuid=30e64d68-b8ee-441a-80e5-fbed7fd5500f"] }], "mendeley" : { "formattedCitation" : "(Isom and Dohlman, 2015)", "plainTextFormattedCitation" : "(Isom and Dohlman, 2015)", "previouslyFormattedCitation" : "(Isom and Dohlman, 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. In the apo state, our calculations in m2r indicate that D103$^{3.32}$ is generally protonated (pK_a = 11.2±1.7), whereas upon ligand binding the pK_a is substantially lowered (pK_a = 7.6±1.9). A possible protonation change of D$^{3.32}$, induced by ligand binding, could thus facilitate the shuttling of protons to D$^{2.50}$. Furthermore, if a G-protein complex with a receptor is preformed before agonist binding, D$^{2.50}$ would be readily accessible for protonation from the extracellular side via a hydrated pathway. In this context, it has further been argued that bound agonists, but not antagonists,

may sustain the hydrated pathway past the ligand which connects the extracellular space with the Na^+ ion binding site upon receptor activation {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/anie.201603766", "ISSN" : "14337851", "author" : [{ "dropping-particle" : "", "family" : "Yuan", "given" : "Shuguang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Peng", "given" : "Qian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palczewski", "given" : "Krzysztof", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vogel", "given" : "Horst", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filipek", "given" : "Slawomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Angewandte Chemie International Edition", "id" : "ITEM-1", "issue" : "30", "issued" : { "date-parts" : [["2016", "7", "18"]] }, "page" : "8661-8665", "title" : "Mechanistic Studies on the Stereoselectivity of the Serotonin 5-HT 1A Receptor", "type" : "article-journal", "volume" : "55" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9e6beb21-d6a0-46a9-ae07-ae8726b9470d"] }], "mendeley" : { "formattedCitation" : "(Yuan et al., 2016)", "plainTextFormattedCitation" : "(Yuan et al., 2016)", "previouslyFormattedCitation" : "(Yuan et al., 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }}. Interestingly, the protonation state of $D69^{2.50}$ shows an effect on the stability of the activated receptor state in our simulations. Under equilibrium, the active state remains stable when $D69^{2.50}$ is neutral (Fig S2), while it exhibits a greater propensity to revert back to the inactive state when $D^{2.50}$ is charged. We obtain similar results for non-equilibrium simulations (Fig S2). This lends further support to an important role of $D^{2.50}$ protonation for receptor activation.

Simulations under electrochemical gradient show ion movement to the intracellular face

Next we conducted atomistic simulations with the Computational Electrophysiology (CompEL) protocol {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.bbamem.2016.02.006", "ISSN" : "0005-2736", "PMID" : "26874204", "author" : [{ "dropping-particle" : "", "family" : "Kutzner", "given" : "Carsten", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-

particle" : "", "family" : "K\u00f6pfer", "given" : "David A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "De", "family" : "Groot", "given" : "Bert L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Song", "given" : "Chen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "BBA - Biomembranes", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016"]] }, "publisher" : "Elsevier B.V.", "title" : "Biochimica et Biophysica Acta Insights into the function of ion channels by computational electrophysiology simulations", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ce24bd41-db25-4edc-8be2-f11184c4f63a"] }], "mendeley" : { "formattedCitation" : "(Kutzner et al., 2016)", "plainTextFormattedCitation" : "(Kutzner et al., 2016)", "previouslyFormattedCitation" : "(Kutzner et al., 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } on the active conformation of m2r. We applied a physiological Na^+ ion gradient of 150:10 mM across the membrane from the extracellular to the intracellular side, in addition to a small ion imbalance evoking a hyperpolarised V_m at -250 mV. Due to the wide range of pK_a values that $D69^{2.50}$ can adopt, its sidechain was modeled both in charged and neutral forms.

Our simulations at -250 mV show that the Na^+ ion exhibits a substantial degree of mobility even when $D69^{2.50}$ is in the charged state (Fig 3 A, B). The Na^+ ion is predominantly coordinated by the residues $D69^{2.50}$, $S110^{3.39}$, $N435^{7.45}$ and $S433^{7.46}$. Under a small membrane voltage, a bimodal distribution of distances between the ion and $D69^{2.50}$ is observed, where larger distances of 5–6 Å are not uncommon (Fig S1). As our pK_a calculations showed that moderate excursions of the ion from its original binding site on this scale are likely to have a major impact on the pK_a and protonation state of the $D69^{2.50}$ sidechain (Fig 2), we investigated the effect of a protonation change of $D69^{2.50}$ in the active conformation.

Our simulations reveal that, in this receptor conformation, the Na^+ ion readily passes through the hydrated channel into the intracellular solution. When $D69^{2.50}$ is neutral, we observe the Na^+ ion to be expelled into the intracellular solution in three out of four simulations at -250 mV (Fig 3 A, C; for a complete list of trajectories see Table S1).

At -500 mV the effect is, expectably, even more pronounced and movement into the cytoplasm is seen in all four simulations we conducted (Fig 3 B, D; Table S1). In contrast, when $D69^{2.50}$ is in a charged state, such a transition is observed only in one out of eight simulations, namely at a raised membrane voltage (Fig 3 A, B; Table S1). The observed translocation of Na^+ to the intracellular side occurs irrespective of the conformation adopted by $Y440^{7.53}$ (Fig 1 D, E and 3 C, D).

In our simulations as well as under physiological conditions, both TM ion concentration and voltage gradients drive ion flow across membrane pores. In the case of the Na^+ ion, both gradients act synergistically in the resting state of the cell, driving the Na^+ ion toward the cytoplasm. Under the conditions used in the simulations, fast ion motion through the receptor is predominantly voltage-driven. Converted into an effective force, and using a linear approximation to describe the gradient across the membrane **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1093/bib/4.4.382", "ISBN" : "9780815344308", "ISSN" : "1467-5463", "abstract" : "What forces drive atoms and molecules to bind, to adsorb, to dissolve, to per- meate membranes, to undergo chemical reactions, and to undergo conforma- tional changes? This is a textbook on statistical thermodynamics. It describes the forces that govern molecular behavior. Statistical thermodynamics uses physical models, mathematical approximations, and empirical laws that are rooted in the language of entropy, distribution function, energy, heat capacity, free energy, and partition function, to predict the behaviors of molecules in physical, chemical, and biological systems. This text is intended for graduate students and advanced undergraduates in physical chemistry, biochemistry, biophysics, bioengineering, polymer and materials science, pharmaceutical chemistry, chemical engineering, and envi- ronmental science. We", "author" : [{ "dropping-particle" : "", "family" : "Dill", "given" : "Ken A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bromberg", "given" : "Sarina", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Statistical Thermodynamics in Chemistry", "id" : "ITEM-1", "issued" : { "date-parts" : [["2011"]] }, "number-of-pages" : "1-661", "title" : "Molecular Driving Force", "type" : "book" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e39cd88a-d2ce-429b-acdb-95629b08ad5d"] }], "mendeley" : { "formattedCitation" : "(Dill and Bromberg, 2011)", "plainTextFormattedCitation" : "(Dill and Bromberg, 2011)", "previouslyFormattedCitation" : "(Dill and Bromberg, 2011)" }, "properties" : {

"noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}**, the influence of the concentration gradient would be about 10-fold smaller (~1.3 pN) than the driving force caused by the voltage gradient under these conditions (~13 pN). At physiological conditions, both driving forces are likely to be of similar magnitude, such that ion migration could either be induced by the voltage or ion gradients.

Energetics of ion movement to the cytoplasm

As the initiation of fast ion movement to the intracellular side was initially tested under slightly supra-physiological levels of V_m, we next evaluated the detailed equilibrium energetics of the Na^+ ion movement on this pathway (i.e. without applied gradients) to ascertain the physiological relevance of this transition. We calculated the potential-of-mean-force (PMF) for the migration of the cation in four different states. In addition to probing the influence of the $D69^{2.50}$ protonation state, we examined the role of the conformation of the $Y440^{7.53}$ sidechain, which substantially affects the width and overall shape of the formed hydrated pathway into the cytoplasm (Fig 3 C, D).

When $D69^{2.50}$ is charged (Fig 4), the free energy difference between the internal Na^+ ion binding site and the free intracellular bulk solution is ~30 kJ mol^{-1}. Accordingly, the active conformation of m2r retains a Na^+ ion at the allosteric site (Z = 7.5-8 Å) with relatively high affinity, as long as $D69^{2.50}$ remains deprotonated. The major barrier to migration into the cytoplasm is located near the $Y440^{7.53}$ sidechain. In its upward state, the free energy barrier amounts to ~41 kJ mol^{-1}, while it increases to ~48 kJ mol^{-1} in the downward state (Fig 4).

As our pK_a calculations showed that even a moderate displacement of the Na^+ ion away from its binding site at $D69^{2.50}$ is likely to lead to a protonation change of the aspartate, we also calculated the PMF for the movement of Na^+ along the intracellular pathway with neutral $D69^{2.50}$. Importantly, this state no longer shows any affinity for the Na^+ ion, and ion movement into the intracellular bulk is not obstructed by any energy barrier significantly larger than the thermal energy (*kT*, ~2.5 kJ mol^{-1}) in the upward-oriented $Y440^{7.53}$ conformation. When $Y440^{7.53}$ is oriented downward, a small but readily surmountable energy barrier (on physiologically relevant timescales) of ~14 kJ mol^{-1} exists for this transition. The downward conformation of $Y440^{7.53}$, in conjunction with the neutral state of $D69^{2.50}$ also has a small influence on the shape and configuration of the ion binding site at $D69^{2.50}$, which leads to a reduction of the number of hydrogen bonds formed between the

protein and the ion (Fig S3), raising the free energy of binding at this site further by ~7.5 kJ mol^{-1} (Fig 4 A, B). In the non-equilibrium case, with a physiological V_m applied, the free energy minima at Z = ~7.5 Å will be raised with regard to the intracellular bulk by ~4.4 kJ mol^{-1} per 100 mV (Fig S4). This means that, in all of these states, the Na^+ ion can readily traverse the receptor and permeate along a hydrated pathway to the intracellular side.

Conservation of the pocket and intracellular exit channel

Additional support for an important role of intracellular Na^+ egress in the activation of class A GPCRs is provided by an analysis of residue conservation along its exit pathway. As we detailed previously {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.tibs.2014.03.002", "ISBN" : "0968-0004", "ISSN" : "09680004", "PMID" : "24767681", "abstract" : "Despite their functional and structural diversity, G-protein-coupled receptors (GPCRs) share a common mechanism of signal transduction via conformational changes in the seven-transmembrane (7TM) helical domain. New major insights into this mechanism come from the recent crystallographic discoveries of a partially hydrated sodium ion that is specifically bound in the middle of the 7TM bundle of multiple class A GPCRs. This review discusses the remarkable structural conservation and distinct features of the Na+ pocket in this most populous GPCR class, as well as the conformational collapse of the pocket upon receptor activation. New insights help to explain allosteric effects of sodium on GPCR agonist binding and activation, and sodium's role as a potential co-factor in class A GPCR function. \u00a9 2014 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Abola", "given" : "Enrique E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Biochemical Sciences", "id" : "ITEM-1", "issue" : "5", "issued" : { "date-parts" : [["2014", "5"]] }, "page" : "233-244", "publisher" : "Elsevier Ltd", "title" : "Allosteric sodium in class A GPCR signaling", "type" : "article", "volume" : "39" }, "uris" : ["http://www.mendeley.com/documents/?uuid=813b6a82-51e4-

47f8-9191-e3f93ac1b280"] }], "mendeley" : { "formattedCitation" : "(Katritch et al., 2014)", "plainTextFormattedCitation" : "(Katritch et al., 2014)", "previouslyFormattedCitation" : "(Katritch et al., 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, there is a remarkable level of conservation for the 16 residues of the Na^+ binding pocket in class A GPCRs (Figure 5, Table S1), suggesting a conserved functional role of Na^+ in receptor activation mechanism. Interestingly, our analysis of Na^+ contacts along the MD trajectories in this study shows that the residues lining the ion exit path to the intracellular side are also well conserved. Thus, out of the 36 contact residues, 32 are 100% conserved among all five muscarinic receptors, 17 are >90% conserved among all aminergic receptors, and 22 are consensus residues among all class A GPCRs. Most importantly, the discovered exit pathway includes Na^+ contacts with the highly conserved $N^{1.50}$ (100% and 98% conserved in aminergic and in all class A respectively), $D^{3.49}$ (100% and 64%), $Y^{5.58}$ (94% and 73%) and other residue positions generally conserved as polar residues, including $N^{1.60}$, $T^{2.37}$ and $N^{2.39}$. Particularly, in the inactive M2 muscarinic receptor and in other inactive state GPCR structures as well, the $Y^{7.53}$ residue is directed toward the Na^+ ion-binding pocket, and hence may play a role as first point of polar contact outside the Na^+ ion-binding pocket for the intracellular movement of Na^+. Na^+ ion passage toward the cytosol may be further facilitated by other conserved polar residues, including $D^{3.49}$, $N^{2.39}$, $N^{2.40}$ and $T^{2.37}$. The conservation of the Na^+ ion pocket and the path for intracellular egress of Na^+ suggests that the Na^+ transfer described in this study can occur in all muscarinic receptors and other class A GPCRs, comprising a key, unidirectional part of the activation mechanism.

DISCUSSION

The principal role of GPCRs is to transmit information about an extracellular agonist binding event toward the cytoplasm, by catalyzing GDP release from a bound intracellular G-protein complex **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nrm908", "ISBN" : "1471-0072 (Print)\\n1471-0072 (Linking)", "ISSN" : "14710072", "PMID" : "12209124", "abstract" : "Seven-transmembrane receptors, which constitute the largest, most ubiquitous and most versatile family of membrane receptors, are also the most common target of therapeutic drugs. Recent findings indicate that the classical models of G-protein coupling and activation of second-messenger-generating enzymes do not fully explain their remarkably diverse

biological actions.", "author" : [{ "dropping-particle" : "", "family" : "Pierce", "given" : "Kristen L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Premont", "given" : "Richard T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lefkowitz", "given" : "Robert J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature reviews. Molecular cell biology", "id" : "ITEM-1", "issue" : "September", "issued" : { "date-parts" : [["2002"]] }, "page" : "639-650", "title" : "Seven-transmembrane receptors.", "type" : "article-journal", "volume" : "3" }, "uris" : ["http://www.mendeley.com/documents/?uuid=203182a1-3741-46b1-8cc5-9251b9c7c01b"] }], "mendeley" : { "formattedCitation" : "(Pierce et al., 2002)", "plainTextFormattedCitation" : "(Pierce et al., 2002)", "previouslyFormattedCitation" : "(Pierce et al., 2002)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. This is known to involve conformational changes in the receptor, including conserved residue microswitches, and large scale movement of TM helices 6 and 7 on the intracellular side that open the nucleotide binding site of the Gα protein **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.sbi.2016.11.005", "ISBN" : "1879-033X (Electronic) 0959-440X (Linking)", "ISSN" : "1879-033X", "PMID" : "27871057", "abstract" : "G protein-coupled receptors (GPCRs) respond to extracellular stimuli and interact with several intracellular binding partners to elicit cellular responses, including heterotrimeric G proteins. Recent structural and biophysical studies have highlighted the dynamic nature of GPCRs and G proteins and have identified specific conformational changes important for receptor-mediated nucleotide exchange on G\u03b1. While domain separation within G\u03b1 is necessary for GDP release, opening the inter-domain interface is insufficient to stimulate nucleotide exchange. Rather, an activated receptor promotes GDP release by allosterically disrupting the nucleotide-binding site via interactions with the G\u03b1 N-termini and C-termini. Highlighting the allosteric nature of GPCRs, recent studies suggest that agonist binding alone poorly stabilizes an active conformation of several receptors. Rather, full stabilization of the receptor in an active state requires formation of the agonist-receptor-G protein ternary complex. In turn, nucleotide-free G\u03b1 is able to stabilize conformational changes around the receptor's agonist-binding site to enhance agonist affinity.", "author" : [{ "dropping-particle" : "", "family" : "Mahoney", "given" : "Jacob P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K.", "non-

dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Current opinion in structural biology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "12"]] }, "page" : "247-254", "publisher" : "Elsevier Ltd", "title" : "Mechanistic insights into GPCR-G protein interactions.", "type" : "article-journal", "volume" : "41" }, "uris" : ["http://www.mendeley.com/documents/?uuid=d2dff388-82b2-4135-98e4-a9b5757d4eb8"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1126/science.aaa5264", "ISSN" : "0036-8075", "author" : [{ "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mildorf", "given" : "Thomas J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hilger", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Borhani", "given" : "David W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Arlow", "given" : "Daniel H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Philippsen", "given" : "Ansgar", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Villanueva", "given" : "Nicolas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yang", "given" : "Zhongyu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lerch", "given" : "Michael T", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hubbell", "given" : "Wayne L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shaw", "given" : "David E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science", "id" : "ITEM-2", "issue" : "6241", "issued" : { "date-parts" : [["2015", "6", "19"]] }, "page" : "1361-1365", "title" : "Structural basis for nucleotide exchange in heterotrimeric G proteins", "type" : "article-journal", "volume" : "348" }, "uris" : ["http://www.mendeley.com/documents/?uuid=cce2ee1a-dc68-4558-aa64-c4ce4f37745c"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1038/nature14886", "ISSN" : "0028-0836", "author" : [{ "dropping-particle" : "", "family" : "Huang", "given" : "Weijiao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-

particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Venkatakrishnan", "given" : "A J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Laeremans", "given" : "Toon", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Feinberg", "given" : "Evan N", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sanborn", "given" : "Adrian L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kato", "given" : "Hideaki E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Livingston", "given" : "Kathryn E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thorsen", "given" : "Thor S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kling", "given" : "Ralf C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Granier", "given" : "S\u00e9bastien", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gmeiner", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Husbands", "given" : "Stephen M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Traynor", "given" : "John R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-3", "issue" : "7565", "issued" : { "date-parts" : [["2015", "8", "5"]] }, "page" : "315-321", "title" : "Structural insights into \u00b5-opioid receptor activation", "type" : "article-journal", "volume" : "524" }, "uris" : ["http://www.mendeley.com/documents/?uuid=25d8632d-4fd4-478d-b0ef-03c07f2f5348"] }], "mendeley" : { "formattedCitation" : "(Dror et al., 2015; Huang et al., 2015; Mahoney and Sunahara, 2016)", "plainTextFormattedCitation" : "(Dror et al., 2015; Huang et al., 2015; Mahoney and Sunahara, 2016)", "previouslyFormattedCitation" : "(Dror et al., 2015; Huang et al., 2015; Mahoney and Sunahara, 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" :

"https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. It has, furthermore, long been recognized that G-protein binding, and stabilization of this conformation on the intracellular side of the receptor, increases agonist affinity on the extracellular face **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "ISSN" : "0026-895X", "PMID" : "4726", "author" : [{ "dropping-particle" : "", "family" : "Maguire", "given" : "M E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Arsdale", "given" : "P M", "non-dropping-particle" : "Van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gilman", "given" : "a G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular pharmacology", "id" : "ITEM-1", "issued" : { "date-parts" : [["1976"]] }, "page" : "335-339", "title" : "An agonist-specific effect of guanine nucleotides on binding to the beta adrenergic receptor.", "type" : "article-journal", "volume" : "12" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9bdf635a-9a7b-44f1-999e-3bda1aac2017"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/nature18324", "ISBN" : "9781617796029", "ISSN" : "1476-4687", "PMID" : "27362234", "abstract" : "G-protein-coupled receptors (GPCRs) remain the primary conduit by which cells detect environmental stimuli and communicate with each other. Upon activation by extracellular agonists, these seven-transmembrane-domain-containing receptors interact with heterotrimeric G proteins to regulate downstream second messenger and/or protein kinase cascades. Crystallographic evidence from a prototypic GPCR, the \u03b22-adrenergic receptor (\u03b22AR), in complex with its cognate G protein, Gs, has provided a model for how agonist binding promotes conformational changes that propagate through the GPCR and into the nucleotide-binding pocket of the G protein \u03b1-subunit to catalyse GDP release, the key step required for GTP binding and activation of G proteins. The structure also offers hints about how G-protein binding may, in turn, allosterically influence ligand binding. Here we provide functional evidence that G-protein coupling to the \u03b22AR stabilizes a \u2018closed\u2019 receptor conformation characterized by restricted access to and egress from the hormone-binding site. Surprisingly, the effects of G protein on the hormone-binding site can be observed in the absence of a bound agonist, where G-protein coupling driven by basal receptor activity impedes the association of agonists, partial agonists, antagonists and inverse agonists. The ability of bound ligands to dissociate from the receptor is also hindered, providing a structural explanation for the G-protein-mediated enhancement of agonist affinity, which has been observed for many GPCR\u2013G-protein pairs. Our data also indicate that, in contrast to agonist binding

alone, coupling of a G protein in the absence of an agonist stabilizes large structural changes in a GPCR. The effects of nucleotide-free G protein on ligand-binding kinetics are shared by other members of the superfamily of GPCRs, suggesting that a common mechanism may underlie G-protein-mediated enhancement of agonist affinity.", "author" : [{ "dropping-particle" : "", "family" : "DeVree", "given" : "Brian T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahoney", "given" : "Jacob P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "V\u00e9lez-Ruiz", "given" : "Gisselle A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rasmussen", "given" : "Soren G. F.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kuszak", "given" : "Adam J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edwald", "given" : "Elin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fung", "given" : "Juan-Jose", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Masureel", "given" : "Matthieu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Du", "given" : "Yang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Matt", "given" : "Rachel A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pardon", "given" : "Els", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-2", "issue" : "7610", "issued" : { "date-parts" : [["2016"]] }, "page" : "182-6", "publisher" : "Nature Publishing Group", "title" : "Allosteric coupling from G protein to the agonist-binding pocket in GPCRs.", "type" : "article-journal", "volume" : "535" }, "uris" : ["http://www.mendeley.com/documents/?uuid=196ee28a-c9cc-497c-b3dc-a8466f7fe7b4"] }], "mendeley" : { "formattedCitation" : "(DeVree et al., 2016; Maguire et al., 1976)", "plainTextFormattedCitation" : "(DeVree et al., 2016; Maguire et al., 1976)",

"previouslyFormattedCitation" : "(DeVree et al., 2016; Maguire et al., 1976)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

Na^+ ions, binding to an internal receptor site between the G-protein and the external ligand binding pockets, are known to act as powerful allosteric modulators of class A GPCRs **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "abstract" : "Receptor binding of the tritiated opiate antagonists naloxone, nalorphine, and levallorphan is enhanced by sodium ion, while binding of the tritiated agonists oxymorphone, dihydromorphine, and levorphanol is diminished. This differential effect of Na+ is highly specific, since it is elicited by Na+ and Li+ but not by other monovalent or divalent cations. The relative effectiveness of nonradioactive opiates in inhibiting [3H]naloxone binding, in the absence and presence of Na+ in vitro, correlates well with their relative agonist antagonist properties in vivo. It is hypothesized that sodium allosterically transforms opiate receptor sites from conformations which bind agonists more readily, to conformations which bind antagonists more readily. This hypothesis is supported by the competition of opiate agonists and antagonists for receptor sites, the marked temperature dependence of binding, the similar extent of binding of tritiated agonists and antagonists at maximal saturation, the concurrent increase in naloxone binding sites and decrease in dihydromorphine binding sites caused by the addition of Na+, and the ability of Na+ to increase [3H] dihydromorphine dissociation, with no effect on [3H]naloxone dissociation.", "author" : [{ "dropping-particle" : "", "family" : "Pert", "given" : "Candace B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Synder", "given" : "Solomon H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular pharmacology", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["1974", "4", "1"]] }, "page" : "868-879", "title" : "Opiate Receptor Binding of Agonists and Antagonists Affected Differentially by Sodium", "type" : "article-journal", "volume" : "10" }, "uris" : ["http://www.mendeley.com/documents/?uuid=36170cac-168d-403c-9106-8c9832a9dbd9"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.tibs.2014.03.002", "ISBN" : "0968-0004", "ISSN" : "09680004", "PMID" : "24767681", "abstract" : "Despite their functional and structural diversity, G-protein-coupled receptors (GPCRs) share a common mechanism of signal transduction via conformational changes in the seven-transmembrane (7TM) helical domain. New major insights into this mechanism come from the recent

crystallographic discoveries of a partially hydrated sodium ion that is specifically bound in the middle of the 7TM bundle of multiple class A GPCRs. This review discusses the remarkable structural conservation and distinct features of the Na+ pocket in this most populous GPCR class, as well as the conformational collapse of the pocket upon receptor activation. New insights help to explain allosteric effects of sodium on GPCR agonist binding and activation, and sodium's role as a potential co-factor in class A GPCR function. \u00a9 2014 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Abola", "given" : "Enrique E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Biochemical Sciences", "id" : "ITEM-2", "issue" : "5", "issued" : { "date-parts" : [["2014", "5"]] }, "page" : "233-244", "publisher" : "Elsevier Ltd", "title" : "Allosteric sodium in class A GPCR signaling", "type" : "article", "volume" : "39" }, "uris" : ["http://www.mendeley.com/documents/?uuid=813b6a82-51e4-47f8-9191-e3f93ac1b280"] }], "mendeley" : { "formattedCitation" : "(Katritch et al., 2014; Pert and Synder, 1974)", "plainTextFormattedCitation" : "(Katritch et al., 2014; Pert and Synder, 1974)", "previouslyFormattedCitation" : "(Katritch et al., 2014; Pert and Synder, 1974)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Na^+ was found to selectively diminish the affinity of agonists, but not antagonists, to GPCRs, which can be interpreted as a structural stabilization of the inactive receptor state by the ions **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1371/journal.pone.0092727", "ISSN" : "1932-6203", "PMID" : "24663151", "abstract" : "The \u03b21-adrenoceptor (\u03b21AR) is a G protein-coupled receptor (GPCR) that is activated by the endogenous agonists adrenaline and noradrenaline. We have determined the structure of an ultra-thermostable \u03b21AR mutant bound to the weak partial agonist cyanopindolol to 2.1 \u00c5 resolution. High-quality crystals (100 \u03bcm plates) were grown in lipidic cubic phase without the assistance of a T4 lysozyme or BRIL fusion in

cytoplasmic loop 3, which is commonly employed for GPCR crystallisation. An intramembrane Na+ ion was identified co-ordinated to Asp872.50, Ser1283.39 and 3 water molecules, which is part of a more extensive network of water molecules in a cavity formed between transmembrane helices 1, 2, 3, 6 and 7. Remarkably, this water network and Na+ ion is highly conserved between \u03b21AR and the adenosine A2A receptor (rmsd of 0.3 \u00c5), despite an overall rmsd of 2.4 \u00c5 for all C\u03b1 atoms and only 23% amino acid identity in the transmembrane regions. The affinity of agonist binding and nanobody Nb80 binding to \u03b21AR is unaffected by Na+ ions, but the stability of the receptor is decreased by 7.5\u00b0C in the absence of Na+. Mutation of amino acid side chains that are involved in the co-ordination of either Na+ or water molecules in the network decreases the stability of \u03b21AR by 5-10\u00b0C. The data suggest that the intramembrane Na+ and associated water network stabilise the ligand-free state of \u03b21AR, but still permits the receptor to form the activated state which involves the collapse of the Na+ binding pocket on agonist binding.", "author" : [{ "dropping-particle" : "", "family" : "Miller-Gallacher", "given" : "Jennifer L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nehm\u00e9", "given" : "Rony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Warne", "given" : "Tony", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edwards", "given" : "Patricia C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schertler", "given" : "Gebhard F X", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Leslie", "given" : "Andrew G W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tate", "given" : "Christopher G", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "PloS one", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["2014", "1"]] }, "page" : "e92727", "title" : "The 2.1 \u00c5 Resolution Structure of Cyanopindolol-Bound \u03b21-Adrenoceptor Identifies an Intramembrane Na⁺ Ion that Stabilises the Ligand-Free Receptor.", "type" : "article-journal", "volume" : "9" }, "uris" : ["http://www.mendeley.com/documents/?uuid=af5847ea-2e45-4ed3-a743-fdff299ddb83"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/sj.bjp.0703382", "ISBN" : "1476-5381", "ISSN" : "0007-1188", "PMID" : "10882382", "abstract" : "1. Sodium ions inhibit spontaneous G(i)/G(o)-coupled receptor activity and promote agonist-induced responses in vitro. The effects of sodium on the relative efficacy of opioid agonists for G-protein activation was measured by guanosine-5'-

O-(gamma-(35)S)-triphosphate ([(35)S]-GTPgammaS) binding in membranes from two mu-opioid receptor-containing systems: CHO cells stably transfected with mouse mureceptors (mMOR-CHO cells) and rat thalamus. 2. NaCl inhibited basal [(35)S]-GTPgammaS binding in both systems, and this effect was partially mimicked by KCl. In mMOR-CHO membranes, net [(35)S]-GTPgammaS binding stimulated by partial but not full agonists was inhibited by NaCl with a potency that was inversely proportional to agonist efficacy. Monovalent cations were required for agonist-stimulated [(35)S]-GTPgammaS binding in this system, and increasing NaCl concentrations magnified relative efficacy differences among agonists. 3. In thalamic membranes, which contain a lower receptor:G-protein ratio than mMOR-CHO cells, similar monovalent cation effects were observed, with two exceptions: (1) [(35)S]-GTPgammaS binding stimulated by both full and partial agonists was inhibited by NaCl; and (2) monovalent cations were not required to observe agonist-stimulated [(35)S]-GTPgammaS binding. 4. Basal [(35)S]-GTPgammaS binding stimulated by the absence of monovalent cations resembled that of agonist-stimulated binding and was blocked by pretreatment of mMOR-CHO cells with pertussis toxin. 5. These results indicate that sodium inhibits spontaneous and agonist-occupied mu receptor-mediated G-protein activation in a manner inversely proportional to the efficacy of the agonist, and that spontaneous mu receptor activity and the relative efficacy of partial agonists acting at these receptors are both increased by increases in the stoichiometric ratio of receptors:G-proteins.", "author" : [{ "dropping-particle" : "", "family" : "Selley", "given" : "D E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cao", "given" : "C C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Liu", "given" : "Q", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Childers", "given" : "S R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "British journal of pharmacology", "id" : "ITEM-2", "issue" : "5", "issued" : { "date-parts" : [["2000"]] }, "page" : "987-996", "title" : "Effects of sodium on agonist efficacy for G-protein activation in mu-opioid receptor-transfected CHO cells and rat thalamus.", "type" : "article-journal", "volume" : "130" }, "uris" : ["http://www.mendeley.com/documents/?uuid=95310d86-041c-40ee-b1ac-e6cbe9b26cb8"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1021/bi961163w", "ISSN" : "0006-2960", "PMID" : "8873604", "abstract" : "Control of the balance between receptor activation and inactivation is a prerequisite for seven transmembrane domain (7TM) receptor function. We asked for a mechanism to stabilize the inactive receptor conformation which prevents

agonist-independent receptor activation. Na+ ions have reciprocal effects on agonist versus antagonist interaction with various 7TM receptors. To investigate the Na+ dependence of receptor activation we chose the bradykinin B2 receptor as a prototypic 7TM receptor. Decrease of the intracellular Na+ content from 40 mM to 10 mM of COS-1 cells transiently expressing rat B2 receptors activated the B2 receptor in the absence of agonist as shown by a 3-fold increase in the basal release of inositolphosphates and increased the intrinsic activity of bradykinin to 1.2. In contrast, under increased intracellular Na+ (148 mM) the intrinsic activity of bradykinin decreased to 0.72. When the interaction of Na+ with the B2 receptor was prevented by exchanging a conserved aspartate in transmembrane domain II for asparagine the B2 receptor was also constitutively-activated in the absence of agonist. Agonist-independence B2 receptor activation under decreased intracellular Na+ was similarly observed with primary human fibroblasts endogenously expressing human B2 receptors by a 2.5-fold increase in basal inositolphosphates. Activation of human B2 receptors in the absence of agonist under decreased intracellular Na+ was further evident by an increased basal phosphorylation of the B2 receptor protein. Thus our data suggest that the interaction of Na+ ions with the B2 receptor stabilizes or induces an inactive receptor conformation thereby providing a mechanism to suppress agonist-independent receptor activation in vivo.", "author" : [{ "dropping-particle" : "", "family" : "Quitterer", "given" : "U", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "AbdAlla", "given" : "S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Jarnagin", "given" : "K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "M\u00fcller-Esterl", "given" : "W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biochemistry", "id" : "ITEM-3", "issue" : "41", "issued" : { "date-parts" : [["1996"]] }, "page" : "13368-77", "title" : "Na+ ions binding to the bradykinin B2 receptor suppress agonist-independent receptor activation.", "type" : "article-journal", "volume" : "35" }, "uris" : ["http://www.mendeley.com/documents/?uuid=bef94e04-2750-49d5-ba7a-7951d7395e0e"] }], "mendeley" : { "formattedCitation" : "(Miller-Gallacher et al., 2014; Quitterer et al., 1996; Selley et al., 2000)", "plainTextFormattedCitation" : "(Miller-Gallacher et al., 2014; Quitterer et al., 1996; Selley et al., 2000)", "previouslyFormattedCitation" : "(Miller-Gallacher et al., 2014; Quitterer et al., 1996; Selley et al., 2000)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Accordingly, while receptor X-ray

structures of sufficient resolution crystallized in the inactive state display a Na^+ ion bound to $D^{2.50}$, this binding site is collapsed in active receptor conformations, and ions are not observed **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature14886", "ISSN" : "0028-0836", "author" : [{ "dropping-particle" : "", "family" : "Huang", "given" : "Weijiao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Venkatakrishnan", "given" : "A J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Laeremans", "given" : "Toon", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Feinberg", "given" : "Evan N", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sanborn", "given" : "Adrian L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kato", "given" : "Hideaki E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Livingston", "given" : "Kathryn E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thorsen", "given" : "Thor S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kling", "given" : "Ralf C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Granier", "given" : "S\u00e9bastien", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gmeiner", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Husbands", "given" : "Stephen M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Traynor", "given" : "John R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7565", "issued" : { "date-parts" : [["2015", "8", "5"]] }, "page" : "315-321", "title" : "Structural insights into \u00b5-opioid receptor activation", "type" : "article-journal", "volume" : "524" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=25d8632d-4fd4-478d-b0ef-03c07f2f5348"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.tibs.2014.03.002", "ISBN" : "0968-0004", "ISSN" : "09680004", "PMID" : "24767681", "abstract" : "Despite their functional and structural diversity, G-protein-coupled receptors (GPCRs) share a common mechanism of signal transduction via conformational changes in the seven-transmembrane (7TM) helical domain. New major insights into this mechanism come from the recent crystallographic discoveries of a partially hydrated sodium ion that is specifically bound in the middle of the 7TM bundle of multiple class A GPCRs. This review discusses the remarkable structural conservation and distinct features of the Na+ pocket in this most populous GPCR class, as well as the conformational collapse of the pocket upon receptor activation. New insights help to explain allosteric effects of sodium on GPCR agonist binding and activation, and sodium's role as a potential co-factor in class A GPCR function. \u00a9 2014 Elsevier Ltd.", "author" : [{ "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Abola", "given" : "Enrique E", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Biochemical Sciences", "id" : "ITEM-2", "issue" : "5", "issued" : { "date-parts" : [["2014", "5"]] }, "page" : "233-244", "publisher" : "Elsevier Ltd", "title" : "Allosteric sodium in class A GPCR signaling", "type" : "article", "volume" : "39" }, "uris" : ["http://www.mendeley.com/documents/?uuid=813b6a82-51e4-47f8-9191-e3f93ac1b280"] }], "mendeley" : { "formattedCitation" : "(Huang et al., 2015; Katritch et al., 2014)", "plainTextFormattedCitation" : "(Huang et al., 2015; Katritch et al., 2014)", "previouslyFormattedCitation" : "(Huang et al., 2015; Katritch et al., 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Mutations around the Na^+ ion binding site have a major impact on receptor function in most class A GPCRs **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1126/science.1219218", "ISBN" : "0036-8075", "ISSN" : "1095-9203", "PMID" :

"22798613", "abstract" : "Pharmacological responses of G protein-coupled receptors (GPCRs) can be fine-tuned by allosteric modulators. Structural studies of such effects have been limited due to the medium resolution of GPCR structures. We reengineered the human A(2A) adenosine receptor by replacing its third intracellular loop with apocytochrome b(562)RIL and solved the structure at 1.8 angstrom resolution. The high-resolution structure allowed us to identify 57 ordered water molecules inside the receptor comprising three major clusters. The central cluster harbors a putative sodium ion bound to the highly conserved aspartate residue Asp(2.50). Additionally, two cholesterols stabilize the conformation of helix VI, and one of 23 ordered lipids intercalates inside the ligand-binding pocket. These high-resolution details shed light on the potential role of structured water molecules, sodium ions, and lipids/cholesterol in GPCR stabilization and function.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Wei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chun", "given" : "Eugene", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chubukov", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xu", "given" : "Fei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Gye Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Christopher B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "Adriaan P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science (New York, N.Y.)", "id" : "ITEM-1", "issue" : "6091", "issued" : { "date-parts" : [["2012", "7", "13"]] }, "page" : "232-6", "title" : "Structural basis for allosteric regulation of GPCRs by sodium ions.", "type" : "article-journal", "volume" : "337" }, "uris" : ["http://www.mendeley.com/documents/?uuid=81df11c9-4f2a-4a4f-8c01-0de1af0d5e09"] }

], "mendeley" : { "formattedCitation" : "(Liu et al., 2012)", "plainTextFormattedCitation" : "(Liu et al., 2012)", "previouslyFormattedCitation" : "(Liu et al., 2012)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**(Liu et al., 2012)(Liu et al., 2012)(Liu et al., 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012)(Liu et al. 2012), either completely abolishing G-protein activation, or resulting in constitutive ligand-independent or pathway-biased signaling **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1126/science.1219218", "ISBN" : "0036-8075", "ISSN" : "1095-9203", "PMID" : "22798613", "abstract" : "Pharmacological responses of G protein-coupled receptors (GPCRs) can be fine-tuned by allosteric modulators. Structural studies of such effects have been limited due to the medium resolution of GPCR structures. We reengineered the human A(2A) adenosine receptor by replacing its third intracellular loop with apocytochrome b(562)RIL and solved the structure at 1.8 angstrom resolution. The high-resolution structure allowed us to identify 57 ordered water molecules inside the receptor comprising three major clusters. The central cluster harbors a putative sodium ion bound to the highly conserved aspartate residue Asp(2.50). Additionally, two cholesterols stabilize the conformation of helix VI, and one of 23 ordered lipids intercalates inside the ligand-binding pocket. These high-resolution details shed light on the potential role of structured water molecules, sodium ions, and lipids/cholesterol in GPCR stabilization and function.", "author" : [{ "dropping-particle" : "", "family" : "Liu", "given" : "Wei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chun", "given" : "Eugene", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chubukov", "given" : "Pavel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xu", "given" : "Fei", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Han", "given" : "Gye Won", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Christopher B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

"dropping-particle" : "", "family" : "IJzerman", "given" : "Adriaan P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science (New York, N.Y.)", "id" : "ITEM-1", "issue" : "6091", "issued" : { "date-parts" : [["2012", "7", "13"]] }, "page" : "232-6", "title" : "Structural basis for allosteric regulation of GPCRs by sodium ions.", "type" : "article-journal", "volume" : "337" }, "uris" : ["http://www.mendeley.com/documents/?uuid=81df11c9-4f2a-4a4f-8c01-0de1af0d5e09"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1124/mol.114.095737", "ISSN" : "1521-0111", "author" : [{ "dropping-particle" : "", "family" : "Massink", "given" : "Arnault", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gutierrez-de-Teran", "given" : "H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lenselink", "given" : "Eelke B", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "V.", "family" : "Ortiz Zacarias", "given" : "N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xia", "given" : "Lizi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heitman", "given" : "Laura H", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "IJzerman", "given" : "A. P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular Pharmacology", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2015", "1", "7"]] }, "page" : "305-313", "title" : "Sodium Ion Binding Pocket Mutations and Adenosine A2A Receptor Function", "type" : "article-journal", "volume" : "87" }, "uris" : ["http://www.mendeley.com/documents/?uuid=bf1014bc-5386-4fde-acd3-eea4821cd7de"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1038/nature12944", "ISSN" : "0028-0836", "PMID" : "24413399", "abstract" : "Opioids represent widely prescribed and abused medications, although their signal transduction mechanisms are not well understood. Here we present the 1.8 \u00c5 high-resolution crystal structure of the human \u03b4-opioid receptor (\u03b4-OR), revealing the presence and fundamental role of a sodium ion in mediating allosteric control of receptor functional selectivity and constitutive activity. The

distinctive \u03b4-OR sodium ion site architecture is centrally located in a polar interaction network in the seven-transmembrane bundle core, with the sodium ion stabilizing a reduced agonist affinity state, and thereby modulating signal transduction. Site-directed mutagenesis and functional studies reveal that changing the allosteric sodium site residue Asn 131 to an alanine or a valine augments constitutive \u03b2-arrestin-mediated signalling. Asp95Ala, Asn310Ala and Asn314Ala mutations transform classical \u03b4-opioid antagonists such as naltrindole into potent \u03b2-arrestin-biased agonists. The data establish the molecular basis for allosteric sodium ion control in opioid signalling, revealing that sodium-coordinating residues act as 'efficacy switches' at a prototypic G-protein-coupled receptor.", "author" : [{ "dropping-particle" : "", "family" : "Fenalti", "given" : "Gustavo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Giguere", "given" : "Patrick M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huang", "given" : "Xi-Ping", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Thompson", "given" : "Aaron a", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roth", "given" : "Bryan L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-3", "issue" : "7487", "issued" : { "date-parts" : [["2014", "2", "13"]] }, "page" : "191-196", "publisher" : "Nature Publishing Group", "title" : "Molecular control of \u03b4-opioid receptor signalling", "type" : "article-journal", "volume" : "506" }, "uris" : ["http://www.mendeley.com/documents/?uuid=317fa7b9-9020-4478-801c-26c164d4fc45"] }], "mendeley" : { "formattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)", "plainTextFormattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)", "previouslyFormattedCitation" : "(Fenalti et al., 2014; Liu et al., 2012; Massink et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

Our work shows that the Na^+ ion binding pocket, which is accessible only from the extracellular face in the inactive state **{**ADDIN CSL_CITATION { "citationItems" : [{ "id"

: "ITEM-1", "itemData" : { "DOI" : "10.1371/journal.pcbi.1000884", "ISSN" : "1553734X", "PMID" : "20711351", "abstract" : "G-protein coupled receptors, the largest family of proteins in the human genome, are involved in many complex signal transduction pathways, typically activated by orthosteric ligand binding and subject to allosteric modulation. Dopaminergic receptors, belonging to the class A family of G-protein coupled receptors, are known to be modulated by sodium ions from an allosteric binding site, although the details of sodium effects on the receptor have not yet been described. In an effort to understand these effects, we performed microsecond scale all-atom molecular dynamics simulations on the dopaminergic D(2) receptor, finding that sodium ions enter the receptor from the extracellular side and bind at a deep allosteric site (Asp2.50). Remarkably, the presence of a sodium ion at this allosteric site induces a conformational change of the rotamer toggle switch Trp6.48 which locks in a conformation identical to the one found in the partially inactive state of the crystallized human beta(2) adrenergic receptor. This study provides detailed quantitative information about binding of sodium ions in the D(2) receptor and reports a possibly important sodium-induced conformational change for modulation of D(2) receptor function.", "author" : [{ "dropping-particle" : "", "family" : "Selent", "given" : "Jana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sanz", "given" : "Ferran", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pastor", "given" : "Manuel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fabritiis", "given" : "Gianni", "non-dropping-particle" : "de", "parse-names" : false, "suffix" : "" }], "container-title" : "PLoS Computational Biology", "id" : "ITEM-1", "issue" : "8", "issued" : { "date-parts" : [["2010", "1"]] }, "title" : "Induced effects of sodium ions on dopaminergic G-protein coupled receptors", "type" : "article-journal", "volume" : "6" }, "uris" : ["http://www.mendeley.com/documents/?uuid=3096738b-0f21-42af-b140-29f4c4df61a6"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.str.2016.04.007", "ISSN" : "09692126", "PMID" : "27210286", "abstract" : "G-protein-coupled receptors (GPCRs) form the largest superfamily of membrane proteins and one-third of all drug targets in humans. A number of recent studies have reported evidence for substantial voltage regulation of GPCRs. However, the structural basis of GPCR voltage sensing has remained enigmatic. Here, we present atomistic simulations on the \u03b4-opioid and M2 muscarinic receptors, which suggest a structural and mechanistic explanation for the observed voltage-induced functional effects. The simulations reveal that the position of an internal Na(+) ion,

recently detected to bind to a highly conserved aqueous pocket in receptor crystal structures, strongly responds to voltage changes. The movements give rise to gating charges in excellent agreement with previous experimental recordings. Furthermore, free energy calculations show that these rearrangements of Na(+) can be induced by physiological membrane voltages. Due to its role in receptor function and signal bias, the repositioning of Na(+) has important general implications for signal transduction in GPCRs.", "author" : [{ "dropping-particle" : "", "family" : "Vickery", "given" : "Owen N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tamburrino", "given" : "Giulia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Seeliger", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Structure", "id" : "ITEM-2", "issue" : "6", "issued" : { "date-parts" : [["2016", "6", "7"]] }, "page" : "997-1007", "publisher" : "The Authors", "title" : "Structural Mechanisms of Voltage Sensing in G Protein-Coupled Receptors", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e4993872-c709-4009-908d-f8916e2440d4"] }], "mendeley" : { "formattedCitation" : "(Selent et al., 2010; Vickery et al., 2016a)", "plainTextFormattedCitation" : "(Selent et al., 2010; Vickery et al., 2016a)", "previouslyFormattedCitation" : "(Selent et al., 2010; Vickery et al., 2016a)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, is transformed into a fully permeable, water-filled channel in the activated receptor conformation of m2r (Fig S5). This channel bridges the extracellular ligand and intracellular G-protein binding sites. Water access from the ligand binding site all the way to the cytoplasmic side of the receptor has previously also been observed in simulations on the $A_{2A}R$ and 5-HT_{1A} receptors **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/ncomms5733", "ISSN" : "2041-1723", "author" : [{ "dropping-particle" : "", "family" : "Yuan", "given" : "Shuguang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filipek", "given" : "Slawomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palczewski", "given" : "Krzysztof", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vogel", "given" : "Horst", "non-dropping-particle" : "",

"parse-names" : false, "suffix" : "" }], "container-title" : "Nature Communications", "id" : "ITEM-1", "issue" : "May", "issued" : { "date-parts" : [["2014"]] }, "page" : "4733", "publisher" : "Nature Publishing Group", "title" : "Activation of G-protein-coupled receptors correlates with the formation of a continuous internal water pathway", "type" : "article-journal", "volume" : "5" }, "uris" : ["http://www.mendeley.com/documents/?uuid=fb3a2654-15f5-430a-947a-d743d513e2bb"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1002/anie.201603766", "ISSN" : "14337851", "author" : [{ "dropping-particle" : "", "family" : "Yuan", "given" : "Shuguang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Peng", "given" : "Qian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palczewski", "given" : "Krzysztof", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vogel", "given" : "Horst", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filipek", "given" : "Slawomir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Angewandte Chemie International Edition", "id" : "ITEM-2", "issue" : "30", "issued" : { "date-parts" : [["2016", "7", "18"]] }, "page" : "8661-8665", "title" : "Mechanistic Studies on the Stereoselectivity of the Serotonin 5-HT 1A Receptor", "type" : "article-journal", "volume" : "55" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9e6beb21-d6a0-46a9-ae07-ae8726b9470d"] }], "mendeley" : { "formattedCitation" : "(Yuan et al., 2014, 2016)", "plainTextFormattedCitation" : "(Yuan et al., 2014, 2016)", "previouslyFormattedCitation" : "(Yuan et al., 2014, 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . We show here that the activated receptor state permits the Na^+ ion to readily cross the receptor toward the cytoplasmic side without experiencing major energy barriers on its pathway. The high hydration level of this pathway in the active state is thereby an important factor in facilitating ion passage. A correlation between hydration level and ion transfer has previously been demonstrated in the case of ion channels **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1073/pnas.1316969110", "ISBN" : "1091-6490 (Electronic)\\r0027-8424 (Linking)", "ISSN" : "1091-6490", "PMID" : "24101457", "abstract" : "The recent crystal structure of Orai, the pore unit of a calcium release-activated calcium (CRAC) channel, is used as the starting point for molecular dynamics and free-energy calculations designed to probe this channel's conduction

properties. In free molecular dynamics simulations, cations localize preferentially at the extracellular channel entrance near the ring of Glu residues identified in the crystal structure, whereas anions localize in the basic intracellular half of the pore. To begin to understand ion permeation, the potential of mean force (PMF) was calculated for displacing a single Na(+) ion along the pore of the CRAC channel. The computed PMF indicates that the central hydrophobic region provides the major hindrance for ion diffusion along the permeation pathway, thereby illustrating the nonconducting nature of the crystal structure conformation. Strikingly, further PMF calculations demonstrate that the mutation V174A decreases the free energy barrier for conduction, rendering the channel effectively open. This seemingly dramatic effect of mutating a nonpolar residue for a smaller nonpolar residue in the pore hydrophobic region suggests an important role for the latter in conduction. Indeed, our computations show that even without significant channel-gating motions, a subtle change in the number of pore waters is sufficient to reshape the local electrostatic field and modulate the energetics of conduction, a result that rationalizes recent experimental findings. The present work suggests the activation mechanism for the wild-type CRAC channel is likely regulated by the number of pore waters and hence pore hydration governs the conductance.", "author" : [{ "dropping-particle" : "", "family" : "Dong", "given" : "Hao", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fiorin", "given" : "Giacomo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Carnevale", "given" : "Vincenzo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Treptow", "given" : "Werner", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Klein", "given" : "Michael L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Proceedings of the National Academy of Sciences of the United States of America", "id" : "ITEM-1", "issued" : { "date-parts" : [["2013"]] }, "page" : "17332-7", "title" : "Pore waters regulate ion permeation in a calcium release-activated calcium channel.", "type" : "article-journal", "volume" : "110" }, "uris" : ["http://www.mendeley.com/documents/?uuid=7f83f244-100a-497a-b59a-79f4bbbdb265"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.bpj.2012.06.003", "ISBN" : "0006-3495", "ISSN" : "00063495", "PMID" : "22853899", "abstract" : "The theoretical prediction of water drying transitions near nonpolar surfaces has stimulated an intensive search for biological processes exploiting this extreme form of hydrophobicity. Here we quantitatively demonstrate that drying of a hydrophobic constriction is the major determinant of ion

conductance in the GLIC pentameric ion channel. Molecular-dynamics simulations show that in the closed state, the channel conductance is ???12 orders-of-magnitude lower than in the open state. This large drop in conductance is remarkable because even in the functionally closed conformation the pore constriction remains wide enough for the passage of sodium ions, aided by a continuous bridge of ???12 water molecules. However, we find that the free energy cost of hydrating the hydrophobic gate is large, accounting almost entirely for the energetic barrier blocking ion passage. The free energies of transferring a sodium ion into a prehydrated gate in functionally closed and open states differ by only 1.2 kcal/mol, compared to an 11 kcal/mol difference in the costs of hydrating the hydrophobic gate. Conversely, ion desolvation effects play only minor roles in GLIC ion channel gating. Our simulations help rationalize experiments probing the gating kinetics of the nicotinic acetylcholine receptor in response to mutations of pore-lining residues. The molecular character and phase behavior of water should thus be included in quantitative descriptions of ion channel gating. ?? 2012 by the Biophysical Society.", "author" : [{ "dropping-particle" : "", "family" : "Zhu", "given" : "Fangqiang", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hummer", "given" : "Gerhard", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical Journal", "id" : "ITEM-2", "issue" : "2", "issued" : { "date-parts" : [["2012"]] }, "page" : "219-227", "publisher" : "Biophysical Society", "title" : "Drying transition in the hydrophobic gate of the GLIC channel blocks ion conduction", "type" : "article-journal", "volume" : "103" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a8e05f6e-f1c8-4eec-9c39-80187bc41fe9"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1016/S0014-5793(03)01151-7", "ISBN" : "1865275182", "ISSN" : "00145793", "PMID" : "14630324", "abstract" : "Ion channels are gated, i.e. they can switch conformation between a closed and an open state. Molecular dynamics simulations may be used to study the conformational dynamics of ion channels and of simple channel models. Simulations on model nanopores reveal that a narrow (<4 ??) hydrophobic region can form a functionally closed gate in the channel and can be opened by either a small (???1 ??) increase in pore radius or an increase in polarity. Modelling and simulation studies confirm the importance of hydrophobic gating in K channels, and support a model in which hinge-bending of the pore-lining M2 (or S6 in Kv channels) helices underlies channel gating. Simulations of a simple outer membrane protein, OmpA, indicate that a gate may also be formed by interactions of charged side chains within a pore, as is also the case in ClC channels. ?? 2003 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.", "author" : [{

"dropping-particle" : "", "family" : "Beckstein", "given" : "Oliver", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Biggin", "given" : "Philip C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bond", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bright", "given" : "Joanne N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Domene", "given" : "Carmen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Grottesi", "given" : "Alessandro", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Holyoake", "given" : "John", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sansom", "given" : "Mark S P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "FEBS Letters", "id" : "ITEM-3", "issue" : "1", "issued" : { "date-parts" : [["2003"]] }, "page" : "85-90", "title" : "Ion channel gating: Insights via molecular simulations", "type" : "article-journal", "volume" : "555" }, "uris" : ["http://www.mendeley.com/documents/?uuid=0d2cc35c-d640-4562-9507-3149e6303f7e"] }], "mendeley" : { "formattedCitation" : "(Beckstein et al., 2003; Dong et al., 2013; Zhu and Hummer, 2012)", "plainTextFormattedCitation" : "(Beckstein et al., 2003; Dong et al., 2013; Zhu and Hummer, 2012)", "previouslyFormattedCitation" : "(Beckstein et al., 2003; Dong et al., 2013; Zhu and Hummer, 2012)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } . In simulations of the inactive state, by contrast, the application of substantially larger forces seems to be necessary to achieve inward migration of Na^+, as no continuous hydrated channel is formed {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1021/bi5006915", "ISSN" : "15204995", "PMID" : "25073009", "abstract" : "The idea of sodium ions altering G-protein-coupled receptor (GPCR) ligand binding and signaling was first suggested for opioid receptors (ORs) in the 1970s and subsequently extended to other GPCRs. Recently published ultra-high-resolution crystal structures of GPCRs, including that of the \u03b4-OR subtype, have started to shed light on the mechanism underlying sodium control in GPCR signaling by revealing details of the sodium binding site. Whether sodium accesses different receptor subtypes from the extra- or intracellular sides, following similar or different pathways, is still an open question. Earlier experiments in brain homogenates suggested a differential sodium regulation of ligand binding to the three major OR subtypes, in spite of their high degree of sequence similarity.

Intrigued by this possibility, we explored the dynamic nature of sodium binding to \u03b4-OR, \u03bc-OR, and \u03ba-OR by means of microsecond-scale, all-atom molecular dynamics (MD) simulations. Rapid sodium permeation was observed exclusively from the extracellular milieu, and following similar binding pathways in all three ligand-free OR systems, notwithstanding extra densities of sodium observed near nonconserved residues of \u03ba-OR and \u03b4-OR, but not in \u03bc-OR. We speculate that these differences may be responsible for the differential increase in antagonist binding affinity of \u03bc-OR by sodium resulting from specific ligand binding experiments in transfected cells. On the other hand, sodium reduced the level of binding of subtype-specific agonists to all OR subtypes. Additional biased and unbiased MD simulations were conducted using the \u03b4-OR ultra-high-resolution crystal structure as a model system to provide a mechanistic explanation for this experimental observation.", "author" : [{ "dropping-particle" : "", "family" : "Shang", "given" : "Yi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lerouzic", "given" : "Valerie", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schneider", "given" : "Sebastian", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bisignano", "given" : "Paola", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pasternak", "given" : "Gavril W", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Filizola", "given" : "Marta", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biochemistry", "id" : "ITEM-1", "issue" : "31", "issued" : { "date-parts" : [["2014", "7", "29"]] }, "page" : "5140-9", "title" : "Mechanistic insights into the allosteric modulation of opioid receptors by sodium ions", "type" : "article-journal", "volume" : "53" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ffb2098d-c620-4989-9511-aa87f79c0558"] }], "mendeley" : { "formattedCitation" : "(Shang et al., 2014)", "plainTextFormattedCitation" : "(Shang et al., 2014)", "previouslyFormattedCitation" : "(Shang et al., 2014)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

The inward motion of the Na^+ ion is likely facilitated by a protonation change of $D^{2.50}$ from the negatively charged to the neutral form, which we show to occur even upon small displacements of the ion from its equilibrium binding position. Neutralization of $D^{2.50}$ substantially reduces the affinity of the binding site for Na^+ ions. Migration of the ion

toward the cytosol is then driven by the negative membrane voltage and by a greater than 10-fold Na^+ gradient across the cytoplasmic membrane under physiological conditions, both strongly attracting Na^+ ions inward. Indeed, we observe that moderately negative membrane voltages allow fast escape of the allosteric Na^+ ion to the cytoplasm on 10–100 ns timescales in our simulations.

According to our results, conformational changes associated with agonist binding from the extracellular side and/or G-protein binding from the cytoplasm alters the Na^+ site conformation and the dynamics of the Na^+–$D^{2.50}$ pair. This, in turn, leads to a protonation change of this residue, and subsequent egress of the Na^+ ion via a hydrated exit channel to the intracellular side.

We therefore suggest that intracellular Na^+ ion transfer, facilitated by the membrane potential and Na^+ gradient, is a pivotal step during receptor activation. We further hypothesize that this transition traps the receptor in the active state (Fig 6). The loss of Na^+ is associated with receptor activation, and it has been shown that, once activated, GPCRs remain in a prolonged active state, capable of signaling even when the receptors are internalized from the cytoplasmic membrane during endocytosis **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.cell.2016.07.004", "ISBN" : "1097-4172 (Electronic) 0092-8674 (Linking)", "ISSN" : "10974172", "PMID" : "27499021", "abstract" : "Classically, G protein-coupled receptor (GPCR) stimulation promotes G protein signaling at the plasma membrane, followed by rapid ??-arrestin-mediated desensitization and receptor internalization into endosomes. However, it has been demonstrated that some??GPCRs activate G proteins from within internalized cellular compartments, resulting in sustained signaling. We have used a variety of biochemical, biophysical, and cell-based methods to demonstrate the existence, functionality, and architecture of internalized receptor complexes composed of a single GPCR, ??-arrestin, and G protein. These super-complexes or ???megaplexes??? more readily form at receptors that interact strongly with ??-arrestins via a C-terminal tail containing clusters of serine/threonine phosphorylation sites. Single-particle electron microscopy analysis of negative-stained purified megaplexes reveals that a single receptor simultaneously binds through its core region with G protein and through its phosphorylated C-terminal tail with ??-arrestin. The formation of such megaplexes provides a potential physical basis for the newly appreciated sustained G protein signaling from internalized GPCRs.", "author" : [{ "dropping-particle" : "", "family" : "Thomsen", "given" : "Alex R B", "non-dropping-

particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Plouffe", "given" : "Bianca", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cahill", "given" : "Thomas J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shukla", "given" : "Arun K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tarrasch", "given" : "Jeffrey T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dosey", "given" : "Annie M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kahsai", "given" : "Alem W.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Strachan", "given" : "Ryan T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pani", "given" : "Biswaranjan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahoney", "given" : "Jacob P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huang", "given" : "Liyin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Breton", "given" : "Billy", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Heydenreich", "given" : "Franziska M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Skiniotis", "given" : "Georgios", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bouvier", "given" : "Michel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lefkowitz", "given" : "Robert J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Cell", "id" : "ITEM-1", "issue" : "4", "issued" : { "date-parts" : [["2016"]] }, "page" : "907-919", "publisher" : "Elsevier Inc.", "title" : "GPCR-G Protein-B-Arrestin Super-Complex Mediates Sustained G Protein Signaling", "type" : "article-journal", "volume" : "166" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c41c1a7c-9dde-483e-ae75-74418f5cde35"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1038/nature12000", "ISBN" : "1476-4687 (Electronic) 0028-0836 (Linking)", "ISSN" : "0028-0836", "PMID" : "23515162", "abstract" : "A long-held tenet of molecular pharmacology is that canonical signal transduction mediated by G-protein-coupled receptor (GPCR) coupling to heterotrimeric G proteins is confined to the plasma membrane.

Evidence supporting this traditional view is based on analytical methods that provide limited or no subcellular resolution. It has been subsequently proposed that signalling by internalized GPCRs is restricted to G-protein-independent mechanisms such as scaffolding by arrestins, or GPCR activation elicits a discrete form of persistent G protein signalling, or that internalized GPCRs can indeed contribute to the acute G-protein-mediated response. Evidence supporting these various latter hypotheses is indirect or subject to alternative interpretation, and it remains unknown if endosome-localized GPCRs are even present in an active form. Here we describe the application of conformation-specific single-domain antibodies (nanobodies) to directly probe activation of the \u03b22-adrenoceptor, a prototypical GPCR, and its cognate G protein, Gs (ref. 12), in living mammalian cells. We show that the adrenergic agonist isoprenaline promotes receptor and G protein activation in the plasma membrane as expected, but also in the early endosome membrane, and that internalized receptors contribute to the overall cellular cyclic AMP response within several minutes after agonist application. These findings provide direct support for the hypothesis that canonical GPCR signalling occurs from endosomes as well as the plasma membrane, and suggest a versatile strategy for probing dynamic conformational change in vivo.", "author" : [{ "dropping-particle" : "", "family" : "Irannejad", "given" : "Roshanak", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tomshine", "given" : "Jin C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tomshine", "given" : "Jon R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chevalier", "given" : "Michael", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahoney", "given" : "Jacob P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rasmussen", "given" : "S\u00f8ren G. F.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sunahara", "given" : "Roger K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "El-Samad", "given" : "Hana", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Huang", "given" : "Bo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zastrow", "given" : "Mark", "non-dropping-particle" : "von", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-2", "issue" : "7442", "issued" : { "date-parts" : [["2013"]]

}, "page" : "534-538", "publisher" : "Nature Publishing Group", "title" : "Conformational biosensors reveal GPCR signalling from endosomes", "type" : "article-journal", "volume" : "495" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f609f0ed-8c27-465a-b9d1-c5e37d8d1b3c"] }], "mendeley" : { "formattedCitation" : "(Irannejad et al., 2013; Thomsen et al., 2016)", "plainTextFormattedCitation" : "(Irannejad et al., 2013; Thomsen et al., 2016)", "previouslyFormattedCitation" : "(Irannejad et al., 2013; Thomsen et al., 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. The crucial role of the Na^+ ion movement within the receptor is reflected by the nearly complete conservation of the Na^+ ion binding site in class A GPCRs, as well as the high conservation level of the exit pathway. The mechanism suggested here is also consistent with agonist independent basal signaling of GPCRs **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.tips.2007.06.003", "ISBN" : "0165-6147", "ISSN" : "01656147", "PMID" : "17629961", "abstract" : "G-protein-coupled receptors (GPCRs) are remarkably versatile signaling molecules. Members of this large family of membrane proteins respond to structurally diverse ligands and mediate most transmembrane signal transduction in response to hormones and neurotransmitters, and in response to the senses of sight, smell and taste. Individual GPCRs can signal through several G-protein subtypes and through G-protein-independent pathways, often in a ligand-specific manner. This functional plasticity can be attributed to structural flexibility of GPCRs and the ability of ligands to induce or to stabilize ligand-specific conformations. Here, we review what has been learned about the dynamic nature of the structure and mechanism of GPCR activation, primarily focusing on spectroscopic studies of purified human ??2 adrenergic receptor. ?? 2007 Elsevier Ltd. All rights reserved.", "author" : [{ "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Deupi", "given" : "Xavier", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Pharmacological Sciences", "id" : "ITEM-1", "issue" : "8", "issued" : { "date-parts" : [["2007"]] }, "page" : "397-406", "title" : "Conformational complexity of G-protein-coupled receptors", "type" : "article-journal", "volume" : "28" }, "uris" : ["http://www.mendeley.com/documents/?uuid=eaf9b8b5-5c57-42ad-b4a1-63888e6e498e"] }], "mendeley" : { "formattedCitation" : "(Kobilka and Deupi, 2007)", "plainTextFormattedCitation" : "(Kobilka and Deupi, 2007)", "previouslyFormattedCitation" : "(Kobilka and Deupi, 2007)" }, "properties" : { "noteIndex"

: 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, explaining this phenomenon as spontaneous protonation of $D^{2.50}$ and egress of the bound Na^+ ion on the intracellular pathway, leading to receptor activation. Following arrival on the cytoplasmic side, it is conceivable that the ion induces further conformational transitions through its strong interaction with protein residues, including at the G-protein-receptor interface and the G-protein itself. This region includes a number of charged and polar groups, for example a polar network extending across all G-proteins, similar to the one observed in GPCRs which enables ion movement **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1073/pnas.1417888112", "ISSN" : "0027-8424", "author" : [{ "dropping-particle" : "", "family" : "Isom", "given" : "Daniel G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dohlman", "given" : "Henrik G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Proceedings of the National Academy of Sciences", "id" : "ITEM-1", "issued" : { "date-parts" : [["2015"]] }, "page" : "201417888", "title" : "Buried ionizable networks are an ancient hallmark of G protein-coupled receptor activation", "type" : "article-journal", "volume" : "2015" }, "uris" : ["http://www.mendeley.com/documents/?uuid=30e64d68-b8ee-441a-80e5-fbed7fd5500f"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.molcel.2013.07.012", "ISBN" : "1097-4164 (Electronic)\\r1097-2765 (Linking)", "ISSN" : "10972765", "PMID" : "23954348", "abstract" : "In response to environmental stress, cells often generate pH signals that serve to protect vital cellular components and reprogram gene expression for survival. A major barrier to our understanding of this process has been the identification of signaling proteins that detect changes in intracellular pH. To identify candidate pH sensors, we developed a computer algorithm that searches proteins for networks of proton-binding sidechains. This analysis indicates that G?? subunits, the principal transducers of G protein-coupled receptor (GPCR) signals, are pH sensors. Our structure-based calculations and biophysical investigations reveal that G?? subunits contain networks of pH-sensing sidechains buried between their Ras and helical domains. Further, we show that proton binding induces changes in conformation that promote G?? phosphorylation and suppress receptor-initiated signaling. Together, our computational, biophysical, and cellular analyses reveal an unexpected function for G proteins as mediators of stress-response signaling. ?? 2013 Elsevier Inc.", "author" : [{ "dropping-particle" : "", "family" : "Isom", "given" : "DanielG", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sridharan", "given" : "Vishwajith", "non-dropping-particle" : "",

"parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Baker", "given" : "Rachael", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Clement", "given" : "SarahT", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Smalley", "given" : "DavidM", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dohlman", "given" : "HenrikG", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Molecular Cell", "id" : "ITEM-2", "issue" : "4", "issued" : { "date-parts" : [["2013"]] }, "page" : "531-538", "publisher" : "Elsevier Inc.", "title" : "Protons as second messenger regulators of G protein signaling", "type" : "article-journal", "volume" : "51" }, "uris" : ["http://www.mendeley.com/documents/?uuid=5e87ba95-d58f-4bc7-b1dc-a6b068e244d7"] }], "mendeley" : { "formattedCitation" : "(Isom and Dohlman, 2015; Isom et al., 2013)", "plainTextFormattedCitation" : "(Isom and Dohlman, 2015; Isom et al., 2013)", "previouslyFormattedCitation" : "(Isom and Dohlman, 2015; Isom et al., 2013)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

Charge movements within membrane proteins, such as the coupled transfer of Na^+ ions and protons suggested by our MD simulations and pK_a calculations, should be sensitive to the membrane voltage. Indeed, it has been demonstrated that GPCR signaling is modulated by membrane voltage changes **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.str.2016.04.007", "ISSN" : "09692126", "PMID" : "27210286", "abstract" : "G-protein-coupled receptors (GPCRs) form the largest superfamily of membrane proteins and one-third of all drug targets in humans. A number of recent studies have reported evidence for substantial voltage regulation of GPCRs. However, the structural basis of GPCR voltage sensing has remained enigmatic. Here, we present atomistic simulations on the \u03b4-opioid and M2 muscarinic receptors, which suggest a structural and mechanistic explanation for the observed voltage-induced functional effects. The simulations reveal that the position of an internal Na(+) ion, recently detected to bind to a highly conserved aqueous pocket in receptor crystal structures, strongly responds to voltage changes. The movements give rise to gating charges in excellent agreement with previous experimental recordings. Furthermore, free energy calculations show that these rearrangements of Na(+) can be induced by physiological membrane voltages. Due to its role in receptor function and signal bias, the repositioning of Na(+) has important general

implications for signal transduction in GPCRs.", "author" : [{ "dropping-particle" : "", "family" : "Vickery", "given" : "Owen N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tamburrino", "given" : "Giulia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Seeliger", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Structure", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["2016", "6", "7"]] }, "page" : "997-1007", "publisher" : "The Authors", "title" : "Structural Mechanisms of Voltage Sensing in G Protein-Coupled Receptors", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e4993872-c709-4009-908d-f8916e2440d4"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1113/jphysiol.2003.056846", "ISSN" : "0022-3751", "PMID" : "14645457", "abstract" : "G-protein-coupled receptor signalling has been suggested to be voltage dependent in a number of cell types; however, the limits of sensitivity of this potentially important phenomenon are unknown. Using the non-excitable rat megakaryocyte as a model system, we now show that P2Y receptor-evoked Ca2+ mobilization is controlled by membrane voltage in a graded and bipolar manner without evidence for a discrete threshold potential. Throughout the range of potentials studied, the peak increase in intracellular Ca2+ concentration ([Ca2+]i) in response to depolarization was always larger than the maximal reduction in [Ca2+]i following an equivalent amplitude hyperpolarization. Significant [Ca2+]i increases were observed in response to small amplitude (< 5 mV, 5 s duration) or short duration (25 ms, 135 mV) depolarizations. Individual cardiac action potential waveforms were also able to repeatedly potentiate P2Y receptor-evoked Ca2+ release and the response to trains of normally paced stimuli fused to generate prolonged [Ca2+]i increases. Furthermore, elevation of the temperature to physiological levels (36 degrees C) resulted in a more sustained depolarization-evoked Ca2+ increase compared with more transient or oscillatory responses at 20-24 degrees C. The ability of signalling via a G-protein-coupled receptor to be potentiated by action potential waveforms and small amplitude depolarizations has broad implications in excitable and non-excitable tissues.", "author" : [{ "dropping-particle" : "", "family" : "Martinez-Pinna", "given" : "Juan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tolhurst", "given" : "Gwen", "non-dropping-particle"

: "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gurung", "given" : "Iman S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vandenberg", "given" : "Jamie I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahaut-Smith", "given" : "Martyn P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of physiology", "id" : "ITEM-2", "issue" : "Pt 1", "issued" : { "date-parts" : [["2004", "2", "15"]] }, "page" : "61-70", "title" : "Sensitivity limits for voltage control of P2Y receptor-evoked Ca²⁺ mobilization in the rat megakaryocyte.", "type" : "article-journal", "volume" : "555" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e1568174-d354-41ba-b815-57c80a3bf202"] }, { "id" : "ITEM-3", "itemData" : { "DOI" : "10.1126/scisignal.aac7419", "ISSN" : "1945-0877", "author" : [{ "dropping-particle" : "", "family" : "Rinne", "given" : "Andreas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mobarec", "given" : "Juan Carlos", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mahaut-Smith", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kolb", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bunemann", "given" : "M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Science Signaling", "id" : "ITEM-3", "issue" : "401", "issued" : { "date-parts" : [["2015", "11", "3"]] }, "page" : "ra110-ra110", "title" : "The mode of agonist binding to a G protein-coupled receptor switches the effect that voltage changes have on signaling", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=f3420ad6-cf1a-485e-8bf7-120a7866c9ae"] }, { "id" : "ITEM-4", "itemData" : { "DOI" : "10.1016/j.tips.2008.05.007", "ISBN" : "0165-6147 (Print)", "ISSN" : "01656147", "PMID" : "18621424", "abstract" : "G-protein-coupled receptors (GPCRs) have ubiquitous roles in transducing extracellular signals into cellular responses. Therefore, the concept that members of this superfamily of surface proteins are directly modulated by changes in membrane voltage could have widespread consequences for cell signalling. Although several studies have indicated that GPCRs can be voltage dependent, particularly P2Y1 receptors in the non-excitable megakaryocyte, the evidence has been mostly indirect. Recent work on muscarinic receptors has stimulated substantial interest in this field by reporting the first voltage-dependent charge movements for a GPCR. An underlying mechanism is proposed whereby a voltage-induced conformational change in

the receptor alters its ability to couple to the G protein and thereby influences its affinity for an agonist. We discuss the strength of the evidence behind this hypothesis and include suggestions for future work. We also describe other examples in which direct voltage control of GPCRs can account for effects of membrane potential on downstream signals and highlight the possible physiological consequences of this phenomenon. ?? 2008 Elsevier Ltd. All rights reserved.", "author" : [{ "dropping-particle" : "", "family" : "Mahaut-Smith", "given" : "Martyn P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Martinez-Pinna", "given" : "Juan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gurung", "given" : "Iman S", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Pharmacological Sciences", "id" : "ITEM-4", "issue" : "8", "issued" : { "date-parts" : [["2008", "8"]] }, "page" : "421-429", "title" : "A role for membrane potential in regulating GPCRs?", "type" : "article", "volume" : "29" }, "uris" : ["http://www.mendeley.com/documents/?uuid=b26de03e-aab3-4f51-82c4-7beab87ec89f"] }, { "id" : "ITEM-5", "itemData" : { "DOI" : "10.1038/nature05259", "ISSN" : "1476-4687", "PMID" : "17065983", "abstract" : "Activation by agonist binding of G-protein-coupled receptors (GPCRs) controls most signal transduction processes. Although these receptors span the cell membrane, they are not considered to be voltage sensitive. Recently it was shown that both the activity of GPCRs and their affinity towards agonists are regulated by membrane potential. However, it remains unclear whether GPCRs intrinsically respond to changes in membrane potential. Here we show that two prototypical GPCRs, the m2 and m1 muscarinic receptors (m2R and m1R), display charge-movement-associated currents analogous to 'gating currents' of voltage-gated channels. The gating charge-voltage relationship of m2R correlates well with the voltage dependence of the affinity of the receptor for acetylcholine. The loop that couples m2R and m1R to their G protein has a crucial function in coupling voltage sensing to agonist-binding affinity. Our data strongly indicate that GPCRs serve as sensors for both transmembrane potential and external chemical signals.", "author" : [{ "dropping-particle" : "", "family" : "Ben-Chaim", "given" : "Yair", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Chanda", "given" : "Baron", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dascal", "given" : "Nathan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bezanilla", "given" : "Francisco", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parnas", "given"

: "Itzchak", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parnas", "given" : "Hanna", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-5", "issue" : "7115", "issued" : { "date-parts" : [["2006", "11", "2"]] }, "page" : "106-9", "title" : "Movement of 'gating charge' is coupled to ligand binding in a G-protein-coupled receptor.", "type" : "article-journal", "volume" : "444" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c37d2319-476d-4482-bc57-24d9e0725fc5"] }, { "id" : "ITEM-6", "itemData" : { "DOI" : "10.1007/s00424-016-1812-y", "ISSN" : "0031-6768", "author" : [{ "dropping-particle" : "", "family" : "Moreno-Galindo", "given" : "Eloy G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Alamilla", "given" : "Javier", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sanchez-Chapula", "given" : "Jos\u00e9 A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tristani-Firouzi", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Navarro-Polanco", "given" : "Ricardo A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Pfl\u00fcgers Archiv - European Journal of Physiology", "id" : "ITEM-6", "issued" : { "date-parts" : [["2016", "3", "29"]] }, "title" : "The agonist-specific voltage dependence of M2 muscarinic receptors modulates the deactivation of the acetylcholine-gated K+ current (I KACh)", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ea663322-34f8-4190-b9ae-add1ec725087"] }], "mendeley" : { "formattedCitation" : "(Ben-Chaim et al., 2006; Mahaut-Smith et al., 2008; Martinez-Pinna et al., 2004; Moreno-Galindo et al., 2016; Rinne et al., 2015; Vickery et al., 2016a)", "plainTextFormattedCitation" : "(Ben-Chaim et al., 2006; Mahaut-Smith et al., 2008; Martinez-Pinna et al., 2004; Moreno-Galindo et al., 2016; Rinne et al., 2015; Vickery et al., 2016a)", "previouslyFormattedCitation" : "(Ben-Chaim et al., 2006; Mahaut-Smith et al., 2008; Martinez-Pinna et al., 2004; Moreno-Galindo et al., 2016; Rinne et al., 2015; Vickery et al., 2016a)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. This applies both to the conformation of the receptors as well as their transmitted signal. Our findings are therefore consistent with these observations, as they suggest that movement of ions in the receptors constitute a key element in the receptor activation process. The observed voltage regulation of GPCRs is of particular relevance for receptors expressed in electrically excitable cells **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-

1", "itemData" : { "DOI" : "10.1016/j.coph.2016.06.004", "ISSN" : "14714892", "PMID" : "27419904", "abstract" : "G-protein coupled receptor (GPCR) modeling approaches are widely used in the hit-to-lead and lead optimization stages of drug discovery. Modern protocols that involve molecular dynamics simulation can address key issues such as the free energy of binding (affinity), ligand-induced GPCR flexibility, ligand binding kinetics, conserved water positions and their role in ligand binding and the effects of mutations. The goals of these calculations are to predict the structures of the complexes between existing ligands and their receptors, to understand the key interactions and to utilize these insights in the design of new molecules with improved binding, selectivity or other pharmacological properties. In this review we present a brief survey of various computational approaches illustrated through a hierarchical GPCR modeling protocol and its prospective application in three industrial drug discovery projects.", "author" : [{ "dropping-particle" : "", "family" : "Heifetz", "given" : "Alexander", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "James", "given" : "Tim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Morao", "given" : "Inaki", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bodkin", "given" : "Michael J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Biggin", "given" : "Philip C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Current Opinion in Pharmacology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "10"]] }, "page" : "14-21", "publisher" : "Elsevier Ltd", "title" : "Guiding lead optimization with GPCR structure modeling and molecular dynamics", "type" : "article-journal", "volume" : "30" }, "uris" : ["http://www.mendeley.com/documents/?uuid=18876317-4439-4ffe-821b-bcec67d9a8a4"] }], "mendeley" : { "formattedCitation" : "(Heifetz et al., 2016)", "plainTextFormattedCitation" : "(Heifetz et al., 2016)", "previouslyFormattedCitation" : "(Heifetz et al., 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. In these cell types, the membrane voltage undergoes large-scale oscillations during action potentials. The transmitted receptor signal could thereby be tuned depending on the specific cell type and its excitation status **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.coph.2016.06.011", "ISSN" : "14714892", "abstract" : "G-protein coupled receptors (GPCRs) form the largest class of membrane proteins in humans and the targets of most present drugs. Membrane potential is one of the defining

characteristics of living cells. Recent work has shown that the membrane voltage, and changes thereof, modulates signal transduction and ligand binding in GPCRs. As it may allow differential signalling patterns depending on tissue, cell type, and the excitation status of excitable cells, GPCR voltage sensitivity could have important implications for their pharmacology. This review summarises recent experimental insights on GPCR voltage regulation and the role of molecular dynamics simulations in identifying the structural basis of GPCR voltage-sensing. We discuss the potential significance for drug design on GPCR targets from excitable and non-excitable cells.", "author" : [{ "dropping-particle" : "", "family" : "Vickery", "given" : "Owen N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Current Opinion in Pharmacology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "10"]] }, "page" : "44-50", "publisher" : "Elsevier Ltd", "title" : "Membrane potentials regulating GPCRs: insights from experiments and molecular dynamics simulations", "type" : "article-journal", "volume" : "30" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c83da8ef-112b-40ed-9da0-8c25a4a56f69"] }], "mendeley" : { "formattedCitation" : "(Vickery et al., 2016b)", "plainTextFormattedCitation" : "(Vickery et al., 2016b)", "previouslyFormattedCitation" : "(Vickery et al., 2016b)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Crucially, many GPCR drug targets are located in excitable tissue in the brain or muscle, where voltage regulation and a differential response to drugs may play an important role.

To summarize, our results suggest a model for class A GPCR activation, in which conformational changes induced by G-protein and agonist binding are accompanied by the intracellular transfer of an internally bound Na^+ ion. Importantly, these conformational changes encompass rearrangement of the sidechain of $Y^{7.53}$, a conserved receptor microswitch **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1146/annurev-pharmtox-032112-135923", "ISSN" : "1545-4304", "PMID" : "23140243", "abstract" : "During the past few years, crystallography of G protein-coupled receptors (GPCRs) has experienced exponential growth, resulting in the determination of the structures of 16 distinct receptors-9 of them in 2012 alone. Including closely related subtype

homology models, this coverage amounts to approximately 12% of the human GPCR superfamily. The adrenergic, rhodopsin, and adenosine receptor systems are also described by agonist-bound active-state structures, including a structure of the receptor-G protein complex for the \u03b2(2)-adrenergic receptor. Biochemical and biophysical techniques, such as nuclear magnetic resonance and hydrogen-deuterium exchange coupled with mass spectrometry, are providing complementary insights into ligand-dependent dynamic equilibrium between different functional states. Additional details revealed by high-resolution structures illustrate the receptors as allosteric machines that are controlled not only by ligands but also by ions, lipids, cholesterol, and water. This wealth of data is helping redefine our knowledge of how GPCRs recognize such a diverse array of ligands and how they transmit signals 30 angstroms across the cell membrane; it also is shedding light on a structural basis of GPCR allosteric modulation and biased signaling.", "author" : [{ "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Annual review of pharmacology and toxicology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2013", "1"]] }, "page" : "531-56", "title" : "Structure-function of the G protein-coupled receptor superfamily.", "type" : "article-journal", "volume" : "53" }, "uris" : ["http://www.mendeley.com/documents/?uuid=16227897-5681-4eba-96d1-07acca5ca220"] }], "mendeley" : { "formattedCitation" : "(Katritch et al., 2013)", "plainTextFormattedCitation" : "(Katritch et al., 2013)", "previouslyFormattedCitation" : "(Katritch et al., 2013)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, which in its upward state allows nearly barrier-free intracellular permeation of Na^+ ions. This observation forms a functional link between the major Na^+ binding site $D^{2.50}$ and $Y^{7.53}$ as the first polar point of contact on the intracellular migration pathway of the Na^+ ion. Translocation of the ion is facilitated by protonation of the conserved $D^{2.50}$ residue (Fig 6) and driven by the physiological membrane Na^+ and voltage gradients. The voltage sensitivity of GPCRs, which has been previously reported for many receptors **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.coph.2016.06.011", "ISSN" : "14714892", "abstract" : "G-protein coupled receptors (GPCRs) form the largest class of membrane proteins in humans and the targets of

most present drugs. Membrane potential is one of the defining characteristics of living cells. Recent work has shown that the membrane voltage, and changes thereof, modulates signal transduction and ligand binding in GPCRs. As it may allow differential signalling patterns depending on tissue, cell type, and the excitation status of excitable cells, GPCR voltage sensitivity could have important implications for their pharmacology. This review summarises recent experimental insights on GPCR voltage regulation and the role of molecular dynamics simulations in identifying the structural basis of GPCR voltage-sensing. We discuss the potential significance for drug design on GPCR targets from excitable and non-excitable cells.", "author" : [{ "dropping-particle" : "", "family" : "Vickery", "given" : "Owen N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Current Opinion in Pharmacology", "id" : "ITEM-1", "issued" : { "date-parts" : [["2016", "10"]] }, "page" : "44-50", "publisher" : "Elsevier Ltd", "title" : "Membrane potentials regulating GPCRs: insights from experiments and molecular dynamics simulations", "type" : "article-journal", "volume" : "30" }, "uris" : ["http://www.mendeley.com/documents/?uuid=c83da8ef-112b-40ed-9da0-8c25a4a56f69"] }], "mendeley" : { "formattedCitation" : "(Vickery et al., 2016b)", "plainTextFormattedCitation" : "(Vickery et al., 2016b)", "previouslyFormattedCitation" : "(Vickery et al., 2016b)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, would thus be a natural consequence of an activation mechanism incorporating the movement of ions as a key element. The Na^+ free receptors are likely to be trapped in an active state, potentially explaining the prolonged mechanisms of signaling observed in many GPCRs. Our results suggest a link between TM signal transduction by receptor proteins and the voltage and ion-gradient driven permeation of ions across ion channels and pores, forming the basis of electric signal transduction in cells.

Based on our findings, we further speculate that the ligand-induced translocation of an ion across the receptor may reflect a common functional principle, which links microbial 7-transmembrane proteins with the structurally remarkably similar eukaryotic GPCRs. The function of microbial 7-transmembrane proteins, such as bacteriorhodopsin and channelrhodopsins, is to transport protons and ions across the membrane following the

absorption of photons {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1021/cr4003769", "ISBN" : "1520-6890 (Electronic)\\r0009-2665 (Linking)", "ISSN" : "00092665", "PMID" : "24364740", "abstract" : "A review. The authors provide mechanistic insights from biophys. and structural studies into the function of microbial and animal rhodopsins, with the latter as representatives of G protein-coupled receptors. [on SciFinder(R)]", "author" : [{ "dropping-particle" : "", "family" : "Ernst", "given" : "Oliver P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lodowski", "given" : "David T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Elstner", "given" : "Marcus", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hegemann", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Brown", "given" : "Leonid S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kandori", "given" : "Hideki", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Chemical Reviews", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2014"]] }, "page" : "126-163", "title" : "Microbial and animal rhodopsins: Structures, functions, and molecular mechanisms", "type" : "article-journal", "volume" : "114" }, "uris" : ["http://www.mendeley.com/documents/?uuid=5aab4322-2cd2-4457-9bb5-718230257c17"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1021/bi027224+", "ISBN" : "0006-2960", "ISSN" : "0006-2960", "PMID" : "12627940", "abstract" : "no abstract", "author" : [{ "dropping-particle" : "", "family" : "Mirzadegan", "given" : "Tara", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Benko", "given" : "Gil", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biochemistry", "id" : "ITEM-2", "issue" : "10", "issued" : { "date-parts" : [["2003"]] }, "page" : "2759-2767", "title" : "Sequence Analyses of G-Protein-Coupled Receptors: Simi- larities to Rhodopsin - Corrections", "type" : "article-journal", "volume" : "42" }, "uris" : ["http://www.mendeley.com/documents/?uuid=d2851562-d719-41eb-b330-33498cb40850"] }], "mendeley" : { "formattedCitation" : "(Ernst et al., 2014; Mirzadegan and Benko, 2003)", "plainTextFormattedCitation" : "(Ernst et al., 2014; Mirzadegan and Benko, 2003)", "previouslyFormattedCitation" : "(Ernst et al., 2014; Mirzadegan and Benko, 2003)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }}.

CONTRIBUTIONS

Conceptualization, V.K., U.Z; Methodology, O.N.V, C.A.C, S.A.Z, A.V.P, V.K, U.Z; Analysis, O.N.V, C.A.C, S.A.Z, A.V.P, V.K, U.Z; Investigation, O.N.V, C.A.C, S.A.Z; Writing – Original draft O.N.V, C.A.C, A.V.P, V.K and U.Z; Writing – Review and editing, O.N.V, C.A.C, S.A.Z, A.V.P, V.K, U.Z; Funding acquisition, V.K. and U.Z; Supervision, V.K. and U.Z.

ACKNOWLEDGEMENTS

This work was supported by the BBSRC (Training Grant BB/J013072/1 to U.Z.) and the Scottish Universities' Physics Alliance (C.A.C, A.V.P. and U.Z.). This research was partially supported by National Institute of Health grant DA035764 to V.K. We thank Salomé Llabrés and Daniel Seeliger for fruitful discussions.

REFERENCES

{ADDIN Mendeley Bibliography CSL_BIBLIOGRAPHY }

Figure Legends

Figure 1: Major structural features and internal hydration of class A GPCRs in the inactive and active state as shown by the m2r. (A) The main structural features of class A GPCRs, as exemplified by m2r, include 7 TM helices (blue), an extracellular ligand binding site, the intracellular effector (G-protein) binding site as well as conserved and functionally important residues termed microswitches (selected ones are highlighted). The vertical axis (Z-coordinate) and all positions stated in the text use the Cα atom of $D103^{3.32}$ as a reference. **(B)** Conformation of inactive m2r (PDB: 3UON) during the simulations showing the presence of the hydrophobic layer separating the hydrophilic pocket and effector binding site. **(C)** After transition to the active state (PDB: 4MQT), and further simulation, m2r displays a continuous water channel connecting the orthosteric ligand binding site, hydrophilic pocket and effector binding site. (**D, E**) The most populated states of the $Y440^{7.53}$ sidechain are demonstrated here in an upward **(D)** and downward conformation **(E)**; please see Fig S3 for a detailed comparison of the upward and downward tyrosine populations. Water molecules are shown in red (surface representation); the position of the allosteric Na^+ ion, as obtained from our initial simulations, is shown as a green sphere, and residues forming the hydrophobic layer (yellow) as well as the bound ligand (carbachol, light green) are depicted in stick representation.

Figure 2: Proximity of the Na^+ ion modulates protonation of $D69^{2.50}$. Continuum electrostatics calculations of the pK_a of the $D69^{2.50}$ sidechain using a multitude of m2r conformations obtained from our atomistic simulations in the carbachol-bound active state, both for $Y440^{7.53}$ in the upward (left) and downward (right) conformations. The pK_a is shown as a function of *Z*, the separation between the Na^+ ion and the C_α atom of $D103^{3.32}$, which marks the orthosteric ligand binding pocket, along the TM axis (see Fig 1A). The data points are in addition coloured according to their distance to the $D69^{2.50}$ sidechain. The black continuous line, a smoothed spline fit, indicates the approximate average pK_a for each separation for illustrative purposes, and the dashed black line shows a pK_a of 7.

Figure 3: Migration of the Na^+ ion across the receptor to the intracellular side.
(A-B) Z-coordinate of the Na^+ ion in m2r under a hyperpolarised V_m of -250 mV (A) and -500 mV (B). Black and grey lines denote simulations with charged $D69^{2.50}$; purple, green and red lines display simulations with neutral $D69^{2.50}$. **(C-D)** Trajectories of the Na^+ ion moving from the hydrophilic pocket, accessible from the extracellular space, into the intracellular bulk solution at -250 mV (C) and -500 mV (D). Three example trajectories are shown for each V_m; please see table S1 for a complete list. The color used to display the Na^+ ion corresponds to the trajectories shown in panels A and B, respectively. Examples of the $Y440^{7.53}$ upward and downward conformations are shown in green. The pathways of the ion toward the intracellular side are almost indistinguishable from each other until the ion passes $Y440^{7.53}$. Thereafter, the pathways diverge to some degree due to the widened exit region to the cytoplasm.

Figure 4: Energetics of Na^+ translocation from the hydrophilic pocket to the intracellular side.
Equilibrium potential of mean force (PMF) profiles of the energetics of Na^+ translocation along the Z-axis in m2r without any applied voltage or concentration gradients. Four relevant states were considered: **(Left)** negatively charged $D69^{2.50}$ (black) or neutral $D69^{2.50}$ (red) with the $Y440^{7.53}$ sidechain in an upward conformation; **(Right)** negatively charged $D69^{2.50}$ (black) or neutral $D69^{2.50}$ (red) with a downward-oriented $Y440^{7.53}$ sidechain. The standard deviation of the PMF, obtained from Bayesian bootstrap analysis, is depicted as shaded area. For each PMF, the intracellular bulk

solution was used as a reference, and the range of positions adopted by the $Y440^{7.53}$ sidechain is denoted by blue dotted lines.

Figure 5: **Conservation of the intracellular Na^+ ion exit pathway.**
The muscarinic m2 receptor is shown in a blue cartoon representation, along with ball-and-stick representation of residues involved in the egress of the Na^+ ion. The carbon atoms of 17 residues that are >90% conserved among aminergic receptors are shown in green, the carbon atoms of additional 15 residues that are conserved among the muscarinic family of receptors are shown in yellow, the carbon atoms of the 4 non-conserved residues are shown in orange.

Figure 6: Proposed role of Na^+ translocation in GPCR activation.
Key checkpoints during the transition from the inactive (A) to active (D) state of the receptor. **(A)** The initial, inactive receptor conformation shows no bound agonist or G-protein, and displays a Na^+ ion bound in a pocket which is sealed towards the cytosol by a hydrophobic layer around $Y^{7.53}$. **(B)** G-protein and agonist bind to the receptor (in undetermined order), leading to the formation of a continuous water channel across the GPCR. The increased mobility of the Na^+ ion results in a pK_a shift and subsequent protonation of $D^{2.50}$. **(C)** Neutralization of $D^{2.50}$ and the presence of the hydrated pathway facilitate transfer of Na^+ to the intracellular side, driven by the transmembrane Na^+ gradient and the negative cytoplasmic membrane voltage. **(D)** The expulsion of Na^+ towards the cytosol results in a prolonged active state of the receptor.

STAR METHODS:

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Ulrich Zachariae, uzachariae@dundee.ac.uk

METHOD DETAILS

System Setup

The simulation system for the m2r in the inactive state was constructed using the crystal structure (PDB: 3UON)**{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature10753", "ISBN" : "1476-4687 (Electronic)\\n0028-0836 (Linking)", "ISSN" : "0028-0836", "PMID" : "22278061", "abstract" : "The parasympathetic branch of the autonomic nervous system regulates the activity of multiple organ systems. Muscarinic receptors are G-protein-coupled receptors that mediate the response to acetylcholine released from parasympathetic nerves. Their role in the unconscious regulation of organ and central nervous system function makes them potential therapeutic targets for a broad spectrum of diseases. The M2 muscarinic acetylcholine

receptor (M2 receptor) is essential for the physiological control of cardiovascular function through activation of G-protein-coupled inwardly rectifying potassium channels, and is of particular interest because of its extensive pharmacological characterization with both orthosteric and allosteric ligands. Here we report the structure of the antagonist-bound human M2 receptor, the first human acetylcholine receptor to be characterized structurally, to our knowledge. The antagonist 3-quinuclidinyl-benzilate binds in the middle of a long aqueous channel extending approximately two-thirds through the membrane. The orthosteric binding pocket is formed by amino acids that are identical in all five muscarinic receptor subtypes, and shares structural homology with other functionally unrelated acetylcholine binding proteins from different species. A layer of tyrosine residues forms an aromatic cap restricting dissociation of the bound ligand. A binding site for allosteric ligands has been mapped to residues at the entrance to the binding pocket near this aromatic cap. The structure of the M2 receptor provides insights into the challenges of developing subtype-selective ligands for muscarinic receptors and their propensity for allosteric regulation.", "author" : [{ "dropping-particle" : "", "family" : "Haga", "given" : "Kazuko", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Asada", "given" : "Hidetsugu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yurugi-Kobayashi", "given" : "Takami", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shiroishi", "given" : "Mitsunori", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhang", "given" : "Cheng", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Okada", "given" : "Tetsuji", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Haga", "given" : "Tatsuya", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobayashi", "given" : "Takuya", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7386", "issued" : { "date-parts" : [["2012", "1", "25"]] }, "page" : "547-551", "publisher" : "Nature Publishing Group", "title" : "Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist",

"type" : "article-journal", "volume" : "482" }, "uris" : ["http://www.mendeley.com/documents/?uuid=aa190004-9d96-48fa-8bf5-f6ce35e7af1b"] }], "mendeley" : { "formattedCitation" : "(Haga et al., 2012)", "plainTextFormattedCitation" : "(Haga et al., 2012)", "previouslyFormattedCitation" : "(Haga et al., 2012)" }, "properties" : { "noteIndex" : 14 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Ligands and non-GPCR subunits were removed. The missing loop ICL3 was modelled using Modeller (v9.14) **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1006/jmbi.1993.1626", "ISBN" : "0022-2836 (Print)\\r0022-2836 (Linking)", "ISSN" : "00222836", "PMID" : "8254673", "abstract" : "We describe a comparative protein modelling method designed to find the most probable structure for a sequence given its alignment with related structures. The three-dimensional (3D) model is obtained by optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. For example, the probabilities for main-chain conformations of a modelled residue may be restrained by its residue type, main-chain conformation of an equivalent residue in a related protein, and the local similarity between the two sequences. Several such pdfs are obtained from the correlations between structural features in 17 families of homologous proteins which have been aligned on the basis of their 3D structures. The pdfs restrain C alpha-C alpha distances, main-chain N-O distances, main-chain and side-chain dihedral angles. A smoothing procedure is used in the derivation of these relationships to minimize the problem of a sparse database. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms. The method is automated and is illustrated by the modelling of trypsin from two other serine proteinases.", "author" : [{ "dropping-particle" : "", "family" : "\u0160ali", "given" : "Andrej", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Blundell", "given" : "Tom L", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Molecular Biology", "id" : "ITEM-1", "issue" : "3", "issued" : { "date-parts" : [["1993", "12"]] }, "page" : "779-815", "title" : "Comparative Protein Modelling by Satisfaction of Spatial Restraints", "type" : "article-journal", "volume" : "234" }, "uris" : [

"http://www.mendeley.com/documents/?uuid=1d6604ad-82cc-4af8-a567-6798efc4da4d"] }], "mendeley" : { "formattedCitation" : "(\u0160ali and Blundell, 1993)", "plainTextFormattedCitation" : "(\u0160ali and Blundell, 1993)", "previouslyFormattedCitation" : "(\u0160ali and Blundell, 1993)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. All internal water molecules and ions were retained, and a Na^+ ion was positioned into the hydrophilic pocket. The charged N- and C-termini were capped using acetyl and methyl moieties, respectively. All ionisable groups were simulated with default protonation states, unless otherwise mentioned. The receptor was embedded into an equilibrated and hydrated 1,2-palmitoyl-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid bilayer using the GROMACS utility g_membed **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcc.21507", "ISBN" : "0192-8651", "ISSN" : "01928651", "PMID" : "20336801", "abstract" : "To efficiently insert a protein into an equilibrated and fully hydrated membrane with minimal membrane perturbation we present a computational tool, called g_membed, which is part of the Gromacs suite of programs. The input consists of an equilibrated membrane system, either flat or curved, and a protein structure in the right position and orientation with respect to the lipid bilayer. g_membed first decreases the width of the protein in the xy-plane and removes all molecules (generally lipids and waters) that overlap with the narrowed protein. Then the protein is grown back to its full size in a short molecular dynamics simulation (typically 1000 steps), thereby pushing the lipids away to optimally accommodate the protein in the membrane. After embedding the protein in the membrane, both the lipid properties and the hydration layer are still close to equilibrium. Thus, only a short equilibration run (less then 1 ns in the cases tested) is required to re-equilibrate the membrane. Its simplicity makes g_membed very practical for use in scripting and high-throughput molecular dynamics simulations.", "author" : [{ "dropping-particle" : "", "family" : "Wolf", "given" : "Maarten G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hoefling", "given" : "Martin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Aponte-Santamar\u00eda", "given" : "Camilo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Grubm\u00fcller", "given" : "Helmut", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Groenhof", "given" : "Gerrit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of

Computational Chemistry", "id" : "ITEM-1", "issued" : { "date-parts" : [["2010"]] }, "page" : "2169-2174", "title" : "G-membed: Efficient insertion of a membrane protein into an equilibrated lipid bilayer with minimal perturbation", "type" : "article-journal", "volume" : "31" }, "uris" : ["http://www.mendeley.com/documents/?uuid=79dc2682-cf05-45ea-9e36-e031f2b031d3"] }], "mendeley" : { "formattedCitation" : "(Wolf et al., 2010)", "plainTextFormattedCitation" : "(Wolf et al., 2010)", "previouslyFormattedCitation" : "(Wolf et al., 2010)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** resulting in a system size of ~92 x 88 x 97 Å. A concentration of 150 mM NaCl in the aqueous solution was used for the single bilayer systems. During equilibration, all protein heavy atoms were position-restrained with a force constant of 1000 kJ mol^{-1} nm^{-2} for 5-10 ns. Due to the low degree of internal hydration and medium resolution of the m2r structure, the equilibration was extended by another 100 ns, now without position restraints, to enable full hydration of the hydrophilic pocket.

Targeted MD

To study the active structure, the ligand carbachol was parameterised using AMBER16, GAFF2 atom types and AM1-BCC partial charges **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Case", "given" : "D", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Betz", "given" : "RM", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Botello-Smith", "given" : "W.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cerutti", "given" : "D.S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cheatham", "given" : "T.E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Darden", "given" : "T.A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Duke", "given" : "R.E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Giese", "given" : "T.J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gohlke", "given" : "H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Goetz", "given" : "A.W.", "non-

dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Homeyer", "given" : "N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Izadi", "given" : "S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Janowski", "given" : "P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kaus", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kovalenko", "given" : "A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lee", "given" : "T.S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "LeGrand", "given" : "S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Li", "given" : "P.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lin", "given" : "C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Luchko", "given" : "T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Luo", "given" : "R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Madej", "given" : "B.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mermelstein", "given" : "D.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Merz", "given" : "K.M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Monard", "given" : "G.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nguyen", "given" : "H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Nguyen", "given" : "H.T.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Omelyan", "given" : "I.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Onufriev", "given" : "A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roe", "given" : "D.R.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Roitberg", "given" : "A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sagui", "given" : "C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Simmerling", "given" : "C.L.", "non-dropping-particle" : "", "parse-names" :

false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Swails", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Walker", "given" : "R.C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wang", "given" : "J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wolf", "given" : "R.M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wu", "given" : "X.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Xiao", "given" : "L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "York", "given" : "D.M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kollman", "given" : "P.A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-1", "issued" : { "date-parts" : [["2016"]] }, "publisher-place" : "University of California, San Francisco", "title" : "Amber 2016", "type" : "article" }, "uris" : ["http://www.mendeley.com/documents/?uuid=8b2814ea-3a37-436f-ab65-a6352ca7f253"] }], "mendeley" : { "formattedCitation" : "(Case et al., 2016)", "plainTextFormattedCitation" : "(Case et al., 2016)", "previouslyFormattedCitation" : "(Case et al., 2016)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, and docked into the orthosteric ligand binding site using GOLD (v5.2.2) **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Willett", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Glen", "given" : "C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-1", "issued" : { "date-parts" : [["1995"]] }, "page" : "43-53", "title" : "Molecular Recognition of Receptor Sites using a Genetic Algorithm with a Description of Desolvation", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a8114da6-d70a-44fe-810a-f773eceb56ec"] }], "mendeley" : { "formattedCitation" : "(Willett and Glen, 1995)", "plainTextFormattedCitation" : "(Willett and Glen, 1995)", "previouslyFormattedCitation" : "(Willett and Glen, 1995)" }, "properties" : { "noteIndex" : 25 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . We then used a targeted MD (TMD) approach with the RMSD to the protein Cα atoms of the active m2r crystal structure (PDB: 4MQT)**{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature12735", "ISSN" : "0028-0836",

"PMID" : "24256733", "abstract" : "Despite recent advances in crystallography and the availability of G-protein-coupled receptor (GPCR) structures, little is known about the mechanism of their activation process, as only the \u03b22 adrenergic receptor (\u03b22AR) and rhodopsin have been crystallized in fully active conformations. Here we report the structure of an agonist-bound, active state of the human M2 muscarinic acetylcholine receptor stabilized by a G-protein mimetic camelid antibody fragment isolated by conformational selection using yeast surface display. In addition to the expected changes in the intracellular surface, the structure reveals larger conformational changes in the extracellular region and orthosteric binding site than observed in the active states of the \u03b22AR and rhodopsin. We also report the structure of the M2 receptor simultaneously bound to the orthosteric agonist iperoxo and the positive allosteric modulator LY2119620. This structure reveals that LY2119620 recognizes a largely pre-formed binding site in the extracellular vestibule of the iperoxo-bound receptor, inducing a slight contraction of this outer binding pocket. These structures offer important insights into the activation mechanism and allosteric modulation of muscarinic receptors.", "author" : [{ "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Ring", "given" : "Aaron M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Manglik", "given" : "Aashish", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Jianxin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hu", "given" : "Kelly", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Eitel", "given" : "Katrin", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "H\u00fcbner", "given" : "Harald", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pardon", "given" : "Els", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Valant", "given" : "Celine", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Sexton", "given" : "Patrick M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Christopoulos", "given" : "Arthur", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Felder", "given" : "Christian C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gmeiner", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false,

"suffix" : "" }, { "dropping-particle" : "", "family" : "Steyaert", "given" : "Jan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Garcia", "given" : "K Christopher", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Wess", "given" : "J\u00fcrgen", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7478", "issued" : { "date-parts" : [["2013", "11", "20"]] }, "page" : "101-106", "title" : "Activation and allosteric modulation of a muscarinic acetylcholine receptor", "type" : "article-journal", "volume" : "504" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ad341a17-7bbd-47c2-8fdc-51826b530f13"] }], "mendeley" : { "formattedCitation" : "(Kruse et al., 2013)", "plainTextFormattedCitation" : "(Kruse et al., 2013)", "previouslyFormattedCitation" : "(Kruse et al., 2013)" }, "properties" : { "noteIndex" : 14 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** as a reference, in order to gently enforce the transition from the inactive (PDB: 3UON)**{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1038/nature10753", "ISBN" : "1476-4687 (Electronic)\\n0028-0836 (Linking)", "ISSN" : "0028-0836", "PMID" : "22278061", "abstract" : "The parasympathetic branch of the autonomic nervous system regulates the activity of multiple organ systems. Muscarinic receptors are G-protein-coupled receptors that mediate the response to acetylcholine released from parasympathetic nerves. Their role in the unconscious regulation of organ and central nervous system function makes them potential therapeutic targets for a broad spectrum of diseases. The M2 muscarinic acetylcholine receptor (M2 receptor) is essential for the physiological control of cardiovascular function through activation of G-protein-coupled inwardly rectifying potassium channels, and is of particular interest because of its extensive pharmacological characterization with both orthosteric and allosteric ligands. Here we report the structure of the antagonist-bound human M2 receptor, the first human acetylcholine receptor to be characterized structurally, to our knowledge. The antagonist 3-quinuclidinyl-benzilate binds in the middle of a long aqueous channel extending approximately two-thirds through the membrane. The orthosteric binding pocket is formed by amino acids that are identical in all five muscarinic receptor subtypes, and shares structural homology with other functionally unrelated acetylcholine binding proteins from

different species. A layer of tyrosine residues forms an aromatic cap restricting dissociation of the bound ligand. A binding site for allosteric ligands has been mapped to residues at the entrance to the binding pocket near this aromatic cap. The structure of the M2 receptor provides insights into the challenges of developing subtype-selective ligands for muscarinic receptors and their propensity for allosteric regulation.", "author" : [{ "dropping-particle" : "", "family" : "Haga", "given" : "Kazuko", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kruse", "given" : "Andrew C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Asada", "given" : "Hidetsugu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Yurugi-Kobayashi", "given" : "Takami", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shiroishi", "given" : "Mitsunori", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zhang", "given" : "Cheng", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Weis", "given" : "William I.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Okada", "given" : "Tetsuji", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobilka", "given" : "Brian K.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Haga", "given" : "Tatsuya", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Kobayashi", "given" : "Takuya", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Nature", "id" : "ITEM-1", "issue" : "7386", "issued" : { "date-parts" : [["2012", "1", "25"]] }, "page" : "547-551", "publisher" : "Nature Publishing Group", "title" : "Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist", "type" : "article-journal", "volume" : "482" }, "uris" : ["http://www.mendeley.com/documents/?uuid=aa190004-9d96-48fa-8bf5-f6ce35e7af1b"] }], "mendeley" : { "formattedCitation" : "(Haga et al., 2012)", "plainTextFormattedCitation" : "(Haga et al., 2012)", "previouslyFormattedCitation" : "(Haga et al., 2012)" }, "properties" : { "noteIndex" : 14 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** to the active state, and further equilibrated it for ~250 ns. While the backbone rapidly transitioned toward the active conformation (Fig. S1), the adaptation of sidechains and the increase in hydration of the receptor occurred on a slightly slower timescale, necessitating this simulation time span. The two major conformations of Y440$^{7.53}$ observed during this simulation were then probed systematically

in the PMF calculations, in which distance restraints between $N^{1.50}$-C_α and $D^{2.50}$-C_α to $Y^{7.53}$-C_ζ and dihedral restraints on the sidechain of $Y^{7.53}$ were used to maintain the protein in the conformations of interest. To keep the G-protein binding site in an active conformation despite the absence of bound G-protein, we applied, at this interaction site, a minimal set of four distance restraints to the C_α atoms of the terminal groups of TM helices 2, 5, 6 and 7, namely between residues 2.39-6.33, 2.39-5.61, 2.43-7.54 and 6.36-7.54 (Fig S6).

CompEL setup

For the CompEL simulations, the aforementioned active state simulation system was duplicated along the Z axis to construct double bilayer systems. A NaCl gradient of 150mM:10mM between the extracellular and intracellular compartments was used, along with an ion imbalance of 1 to 2 Cl^- ions to generate a V_m of ~-250 to ~-500 mV, as previously described **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.bpj.2011.06.010", "ISSN" : "1542-0086", "PMID" : "21843471", "abstract" : "Presently, most simulations of ion channel function rely upon nonatomistic Brownian dynamics calculations, indirect interpretation of energy maps, or application of external electric fields. We present a computational method to directly simulate ion flux through membrane channels based on biologically realistic electrochemical gradients. In close analogy to single-channel electrophysiology, physiologically and experimentally relevant timescales are achieved. We apply our method to the bacterial channel PorB from pathogenic Neisseria meningitidis, which, during Neisserial infection, inserts into the mitochondrial membrane of target cells and elicits apoptosis by dissipating the membrane potential. We show that our method accurately predicts ion conductance and selectivity and elucidates ion conduction mechanisms in great detail. Handles for overcoming channel-related antibiotic resistance are identified.", "author" : [{ "dropping-particle" : "", "family" : "Kutzner", "given" : "Carsten", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Grubm\u00fcller", "given" : "Helmut", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Groot", "given" : "Bert L", "non-dropping-particle" : "de", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical journal", "id" : "ITEM-1", "issue" : "4", "issued" : { "date-parts" : [["2011", "8", "17"]] }, "page" : "809-17", "title" : "Computational electrophysiology: the molecular dynamics of ion channel

permeation and selectivity in atomistic detail.", "type" : "article-journal", "volume" : "101" }, "uris" : ["http://www.mendeley.com/documents/?uuid=8a3dfc96-63fe-4b51-88f6-37ad9c60e90e"] }], "mendeley" : { "formattedCitation" : "(Kutzner et al., 2011)", "plainTextFormattedCitation" : "(Kutzner et al., 2011)", "previouslyFormattedCitation" : "(Kutzner et al., 2011)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. The V_m was determined by the GROMACS utility gmx potential.

Potential of mean force calculations

To calculate the PMF for Na^+ ion translocation across m2r at neutral V_m, umbrella sampling calculations were performed in bins of 0.25 Å and analysed with the GROMACS utility gmx wham. We used a simulation time of 50 ns in each window and harmonic potentials of 900–2000 kJ mol^{-1} nm^{-2} to restrain the Na^+ ion in the Z-direction. The standard deviation of the PMF profiles was estimated by using the Bayesian bootstrap method, as implemented in gmx wham, with 200 runs (See quantification and statistical analysis). The free energy of the Na^+ ion in bulk solution was set to 0. The position of the Na^+ ion (Z-coordinate) is reported relative to the $D103^{3.32}$-C_α atom (ligand binding site). These calculations were performed for the active conformation in both the apo and carbachol-bound state. In the latter case, $D69^{2.50}$ was modelled both in the negatively charged and neutral state with the $Y440^{7.53}$ sidechain in an upward or downward conformation.

Gating charge calculations

To calculate the gating charges, we followed a method previously described in **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.str.2016.04.007", "ISSN" : "09692126", "PMID" : "27210286", "abstract" : "G-protein-coupled receptors (GPCRs) form the largest superfamily of membrane proteins and one-third of all drug targets in humans. A number of recent studies have reported evidence for substantial voltage regulation of GPCRs. However, the structural basis of GPCR voltage sensing has remained enigmatic. Here, we present atomistic simulations on the \u03b4-opioid and M2 muscarinic receptors, which suggest a structural and mechanistic explanation for the observed voltage-induced functional effects. The simulations reveal that the position of an internal Na(+) ion, recently detected to bind to a highly conserved aqueous pocket in receptor crystal structures, strongly responds to voltage changes. The movements give rise to gating charges in excellent agreement with previous experimental recordings.

Furthermore, free energy calculations show that these rearrangements of Na(+) can be induced by physiological membrane voltages. Due to its role in receptor function and signal bias, the repositioning of Na(+) has important general implications for signal transduction in GPCRs.", "author" : [{ "dropping-particle" : "", "family" : "Vickery", "given" : "Owen N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Machtens", "given" : "Jan-Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Tamburrino", "given" : "Giulia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Seeliger", "given" : "Daniel", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Zachariae", "given" : "Ulrich", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Structure", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["2016", "6", "7"]] }, "page" : "997-1007", "publisher" : "The Authors", "title" : "Structural Mechanisms of Voltage Sensing in G Protein-Coupled Receptors", "type" : "article-journal", "volume" : "24" }, "uris" : ["http://www.mendeley.com/documents/?uuid=e4993872-c709-4009-908d-f8916e2440d4"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1016/j.bpj.2017.02.016", "ISSN" : "15420086", "abstract" : "Electrical cell signaling requires adjustment of ion channel, receptor, or transporter function in response to changes in membrane potential. For the majority of such membrane proteins, the molecular details of voltage sensing remain insufficiently understood. Here, we present a molecular dynamics simulation-based method to determine the underlying charge movement across the membrane???the gating charge???by measuring electrical capacitor properties of membrane-embedded proteins. We illustrate the approach by calculating the charge transfer upon membrane insertion of the HIV gp41 fusion peptide, and validate the method on two prototypical voltage-dependent proteins, the Kv1.2 K+ channel and the voltage sensor of the Ciona intestinalis voltage-sensitive phosphatase, against experimental data. We then use the gating charge analysis to study how the T1 domain modifies voltage sensing in Kv1.2 channels and to investigate the voltage dependence of the initial binding of two Na+ ions in Na+-coupled glutamate transporters. Our simulation approach quantifies various mechanisms of voltage sensing, enables direct comparison with experiments, and supports mechanistic interpretation of voltage sensitivity by fractional amino acid contributions.", "author" : [{ "dropping-particle" : "", "family" : "Machtens", "given" : "Jan Philipp", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Briones", "given" : "Rodolfo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

"dropping-particle" : "", "family" : "Alleva", "given" : "Claudia", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Groot", "given" : "Bert L.", "non-dropping-particle" : "de", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fahlke", "given" : "Christoph", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical Journal", "id" : "ITEM-2", "issue" : "7", "issued" : { "date-parts" : [["2017"]] }, "page" : "1396-1405", "publisher" : "Biophysical Society", "title" : "Gating Charge Calculations by Computational Electrophysiology Simulations", "type" : "article-journal", "volume" : "112" }, "uris" : ["http://www.mendeley.com/documents/?uuid=2aa40729-ae4d-4ad6-8d1f-d7696ab42a1f"] }], "mendeley" : { "formattedCitation" : "(Machtens et al., 2017; Vickery et al., 2016a)", "manualFormatting" : "Machtens et al., 2017 and Vickery et al., 2016a)", "plainTextFormattedCitation" : "(Machtens et al., 2017; Vickery et al., 2016a)", "previouslyFormattedCitation" : "(Machtens et al., 2017; Vickery et al., 2016a)" }, "properties" : { "noteIndex" : 15 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. A single bilayer of the active system was duplicated along the Z-axis, with one bilayer inverted (intracellular components of the receptors facing each other). The charge imbalance between compartments was then neutralised by adding ions. All protein atoms except hydrogen atoms were position-restrained using a spring constant of 1000 kJ mol^{-1} nm^{-2}, whilst the Na^+ ion was restrained with a force constant of 10,000 kJ mol^{-1} nm^{-2} due to its greater mobility. Bulk Na^+ ions were position-restrained on the Z-axis using a spring constant of 200 kJ mol^{-1} nm^{-2} to prevent their ingress into the receptor. The systems were calibrated using charge imbalances of -4 to 4; the slopes of the charge imbalance-voltage relationships indicate a near constant capacitance of the membrane/protein system under these conditions. The gating charges were inferred from the voltage differences for each ion position at a given charge imbalance. Errors were derived from the maximum and minimum slopes of the charge imbalance-voltage relationships. The hydrophilic channel was scanned by placing the ion at 2.5 Å intervals from the hydrophilic pocket to the intracellular solution and simulated for 50 ns, with the first 5 ns discarded (Fig S4 E). The gating charge calculated for each interval was taken as a direct measure of the voltage drop within the hydrated channel. This voltage drop, multiplied by the elementary charge *e* for a monovalent ion, was added to the equilibrium PMFs obtained by umbrella sampling (Fig S4 A-D), representing the excess free energy.

Forcefield parameters

For all MD simulations, the amber99sb_ildn force field was used for the protein {ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/prot.22711", "ISBN" : "0887-3585", "ISSN" : "1097-0134", "PMID" : "20408171", "abstract" : "Recent advances in hardware and software have enabled increasingly long molecular dynamics (MD) simulations of biomolecules, exposing certain limitations in the accuracy of the force fields used for such simulations and spurring efforts to refine these force fields. Recent modifications to the Amber and CHARMM protein force fields, for example, have improved the backbone torsion potentials, remedying deficiencies in earlier versions. Here, we further advance simulation accuracy by improving the amino acid side-chain torsion potentials of the Amber ff99SB force field. First, we used simulations of model alpha-helical systems to identify the four residue types whose rotamer distribution differed the most from expectations based on Protein Data Bank statistics. Second, we optimized the side-chain torsion potentials of these residues to match new, high-level quantum-mechanical calculations. Finally, we used microsecond-timescale MD simulations in explicit solvent to validate the resulting force field against a large set of experimental NMR measurements that directly probe side-chain conformations. The new force field, which we have termed Amber ff99SB-ILDN, exhibits considerably better agreement with the NMR data.", "author" : [{ "dropping-particle" : "", "family" : "Lindorff-Larsen", "given" : "Kresten", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Piana", "given" : "Stefano", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Palmo", "given" : "Kim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Maragakis", "given" : "Paul", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Klepeis", "given" : "John L.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Dror", "given" : "Ron O.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Shaw", "given" : "David E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Proteins", "id" : "ITEM-1", "issue" : "8", "issued" : { "date-parts" : [["2010", "6"]] }, "page" : "1950-8", "title" : "Improved side-chain torsion potentials for the Amber ff99SB protein force field.", "type" : "article-journal", "volume" : "78" }, "uris" : ["http://www.mendeley.com/documents/?uuid=47a59efa-af9c-451a-9e52-e1dc5341e8f7"] }], "mendeley" : { "formattedCitation" : "(Lindorff-Larsen et al., 2010)", "plainTextFormattedCitation" : "(Lindorff-Larsen et al., 2010)",

"previouslyFormattedCitation" : "(Lindorff-Larsen et al., 2010)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, Berger parameters for lipids **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/S0006-3495(97)78845-3", "ISBN" : "4687907164", "ISSN" : "00063495", "PMID" : "9129804", "abstract" : "Molecular dynamics simulations of 500 ps were performed on a system consisting of a bilayer of 64 molecules of the lipid dipalmitoylphosphatidylcholine and 23 water molecules per lipid at an isotropic pressure of 1 atm and 50 degrees C. Special attention was devoted to reproduce the correct density of the lipid, because this quantity is known experimentally with a precision better than 1%. For this purpose, the Lennard-Jones parameters of the hydrocarbon chains were adjusted by simulating a system consisting of 128 pentadecane molecules and varying the Lennard-Jones parameters until the experimental density and heat of vaporization were obtained. With these parameters the lipid density resulted in perfect agreement with the experimental density. The orientational order parameter of the hydrocarbon chains agreed perfectly well with the experimental values, which, because of its correlation with the area per lipid, makes it possible to give a proper estimate of the area per lipid of 0.61 +/- 0.01 nm2.", "author" : [{ "dropping-particle" : "", "family" : "Berger", "given" : "Oliver", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Edholm", "given" : "Olle", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "J\u00e4hnig", "given" : "F", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical journal", "id" : "ITEM-1", "issue" : "May 1997", "issued" : { "date-parts" : [["1997"]] }, "page" : "2002-2013", "title" : "Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature.", "type" : "article-journal", "volume" : "72" }, "uris" : ["http://www.mendeley.com/documents/?uuid=99984f9c-f326-4269-b006-450fcb67c57d"] }], "mendeley" : { "formattedCitation" : "(Berger et al., 1997)", "plainTextFormattedCitation" : "(Berger et al., 1997)", "previouslyFormattedCitation" : "(Berger et al., 1997)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, which were adapted for use with the amber99sb force field **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1021/ct200491c", "ISBN" : "1549-9618", "ISSN" : "15499618", "abstract" : "AMBER force fields are among the most

commonly used in molecular dynamics (MD) simulations of proteins. Unfortunately, they lack a specific set of lipid parameters, thus limiting its use in membrane protein simulations. In order to overcome this limitation we assessed whether the widely used united-atom lipid parameters described by Berger and co-workers could be used in conjunction with AMBER force fields in simulations of membrane proteins. Thus, free energies of solvation in water and in cyclohexane, and free energies of water to cyclohexane transfer, were computed by thermodynamic integration procedures for neutral amino acid side-chains employing AMBER99, AMBER03, and OPLS-AA amino acid force fields. In addition, MD simulations of three membrane proteins in a POPC lipid bilayer, the ?2 adrenergic G protein-coupled receptor, Aquaporin-1, and the outer membrane protein Omp32, were performed with the aim of comparing the AMBER99SB/Berger combination of force fields with the OPLS-AA/Berger combination. We have shown that AMBER99SB and Berger force fields are compatible, they provide reliable free energy estimations relative to experimental values, and their combination properly describes both membrane and protein structural properties. We then suggest that the AMBER99SB/Berger combination is a reliable choice for the simulation of membrane proteins, which links the easiness of ligand parametrization and the ability to reproduce secondary structure of AMBER99SB force field with the largely validated Berger lipid parameters.", "author" : [{ "dropping-particle" : "", "family" : "Cordom\u00ed", "given" : "Arnau", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Caltabiano", "given" : "Gianluigi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pardo", "given" : "Leonardo", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Chemical Theory and Computation", "id" : "ITEM-1", "issued" : { "date-parts" : [["2012"]] }, "page" : "948-958", "title" : "Membrane protein simulations using AMBER force field and Berger lipid parameters", "type" : "article-journal", "volume" : "8" }, "uris" : ["http://www.mendeley.com/documents/?uuid=ac4960a4-e27d-44e9-bae8-872ef45d1e25"] }], "mendeley" : { "formattedCitation" : "(Cordom\u00ed et al., 2012)", "plainTextFormattedCitation" : "(Cordom\u00ed et al., 2012)", "previouslyFormattedCitation" : "(Cordom\u00ed et al., 2012)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**, and the SPC/E model for water molecules **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1021/j100308a038", "ISBN" : "0022-3654", "ISSN" : "0022-3654", "abstract" :

"Effective pair potentials used for simulations of polar liquids include the average effects of polarization. Such potentials are generally adjusted to produce the experimental heat of vaporization. It has not been recognized before that the self-energy term inherent in any polarizable model should be included in effective pair potentials as well. Inclusion of the self-energy correction with a consequent reparametrization of the SPC (simple point charge) model of water yields an improvement of the effective pair potential for water, as exemplified by density, radial distribution functions, and diffusion constant.", "author" : [{ "dropping-particle" : "", "family" : "Berendsen", "given" : "H J C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Grigera", "given" : "J R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Straatsma", "given" : "T P", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Physical Chemistry", "id" : "ITEM-1", "issue" : "24", "issued" : { "date-parts" : [["1987"]] }, "page" : "6269-6271", "title" : "The Missing Term in Effective Pair Potentials", "type" : "article-journal", "volume" : "91" }, "uris" : ["http://www.mendeley.com/documents/?uuid=5aad4bf7-8fc6-4d33-b444-2560fe827a9a"] }], "mendeley" : { "formattedCitation" : "(Berendsen et al., 1987)", "plainTextFormattedCitation" : "(Berendsen et al., 1987)", "previouslyFormattedCitation" : "(Berendsen et al., 1987)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. Water bond angles and distances were constrained by SETTLE **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/jcc.540130805", "ISBN" : "0192-8651", "ISSN" : "1096-987X", "PMID" : "8229760", "abstract" : "An analytical algorithm, called SETTLE, for resetting the positions and velocities to satisfy the holonomic constraints on the rigid water model is presented. This method is still based on the Cartesian coordinate system and can be used in place of SHAKE and RATTLE. We implemented this algorithm in the SPASMS package of molecular mechanics and dynamics. Several series of molecular dynamics simulations were car- ried out to examine the performance of the new algorithm in comparison with the original RATTLE method. It was found that SETTLE is of higher accuracy and is faster than RATTLE with reasonable tolerances by three to nine times on a scalar machine. Furthermore, the performance improvement ranged from fac- tors of 26 to 98 on a vector machine since the method presented is not iterative.", "author" : [{ "dropping-particle" : "", "family" : "Miyamoto", "given" : "Shuichi", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, {

"dropping-particle" : "", "family" : "Kollman", "given" : "Peter A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of computational chemistry", "id" : "ITEM-1", "issued" : { "date-parts" : [["1992"]] }, "page" : "952-962", "title" : "SETTLE: an analytical version of the SHAKE and RATTLE algorithm for rigid water models", "type" : "article-journal", "volume" : "13" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a95a290c-4007-4cb6-b91b-74c596ae58c1"] }], "mendeley" : { "formattedCitation" : "(Miyamoto and Kollman, 1992)", "plainTextFormattedCitation" : "(Miyamoto and Kollman, 1992)", "previouslyFormattedCitation" : "(Miyamoto and Kollman, 1992)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** while all other bonds were constrained using the LINCS method **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H", "ISBN" : "0192-8651", "ISSN" : "0192-8651", "abstract" : "In this article, we present a new LINear Constraint Solver (LINCS) for molecular simulations with bond constraints. The algorithm is inherently stable, as the constraints themselves are reset instead of derivatives of the constraints, thereby eliminating drift. Although the derivation of the algorithm is presented in terms of matrices, no matrix matrix multiplications are needed and only the nonzero matrix elements have to be stored, making the method useful for very large molecules. At the same accuracy, the LINCS algorithm is three to four times faster than the SHAKE algorithm. Parallelization of the algorithm is straightforward.\ufffd\ufffd\ufffd\ufffd 1997 John Wiley & Sons, Inc.\ufffd\ufffd\ufffdJ Comput Chem 18: 1463-1472, 1997", "author" : [{ "dropping-particle" : "", "family" : "Hess", "given" : "Berk", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bekker", "given" : "Henk", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Berendsen", "given" : "Herman J. C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Fraaije", "given" : "Johannes G. E. M.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Computational Chemistry", "id" : "ITEM-1", "issue" : "12", "issued" : { "date-parts" : [["1997"]] }, "page" : "1463-1472", "title" : "LINCS: A linear constraint solver for molecular simulations", "type" : "article-journal", "volume" : "18" }, "uris" : ["http://www.mendeley.com/documents/?uuid=72f9cf75-9f09-4442-9759-2599b358e798"] }], "mendeley" : { "formattedCitation" : "(Hess et al., 1997)", "plainTextFormattedCitation"

: "(Hess et al., 1997)", "previouslyFormattedCitation" : "(Hess et al., 1997)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**. The temperature and pressure were kept constant throughout the simulations at 310 K and 1 bar, respectively, with the protein, lipids, and water/ions coupled individually to a temperature bath by the v-rescale method using a time constant of 0.2 ps and a semi-isotropic Berendsen barostat **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1063/1.2408420", "ISBN" : "0021-9606 (Print)\\r0021-9606 (Linking)", "ISSN" : "00219606", "PMID" : "17212484", "abstract" : "The authors present a new molecular dynamics algorithm for sampling the canonical distribution. In this approach the velocities of all the particles are rescaled by a properly chosen random factor. The algorithm is formally justified and it is shown that, in spite of its stochastic nature, a quantity can still be defined that remains constant during the evolution. In numerical applications this quantity can be used to measure the accuracy of the sampling. The authors illustrate the properties of this new method on Lennard-Jones and TIP4P water models in the solid and liquid phases. Its performance is excellent and largely independent of the thermostat parameter also with regard to the dynamic properties.", "author" : [{ "dropping-particle" : "", "family" : "Bussi", "given" : "Giovanni", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Donadio", "given" : "Davide", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Parrinello", "given" : "Michele", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Chemical Physics", "id" : "ITEM-1", "issued" : { "date-parts" : [["2007"]] }, "title" : "Canonical sampling through velocity rescaling", "type" : "article-journal", "volume" : "126" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4218bfcf-f68f-4d8c-ab36-4806cf155f86"] }, { "id" : "ITEM-2", "itemData" : { "DOI" : "10.1063/1.448118", "ISBN" : "doi:10.1063/1.448118", "ISSN" : "00219606", "PMID" : "21370443", "abstract" : "In molecular dynamics (MD) simulations the need often arises to maintain such parameters as temperature or pressure rather than energy and volume, or to impose gradients for studying transport properties in nonequilibrium MD. A method is described to realize coupling to an external bath with constant temperature or pressure with adjustable time constants for the coupling. The method is easily extendable to other variables and to gradients, and can be applied also to polyatomic molecules involving internal constraints. The influence of coupling time constants on dynamical variables is evaluated. A leap-frog algorithm is presented for the general case involving constraints with

coupling to both a constant temperature and a constant pressure bath.", "author" : [{ "dropping-particle" : "", "family" : "Berendsen", "given" : "H J C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Postma", "given" : "J P M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gunsteren", "given" : "W F", "non-dropping-particle" : "van", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "DiNola", "given" : "A", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Haak", "given" : "J R", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "The Journal of Chemical Physics", "id" : "ITEM-2", "issued" : { "date-parts" : [["1984"]] }, "page" : "3684-3690", "title" : "Molecular dynamics with coupling to an external bath", "type" : "article-journal", "volume" : "81" }, "uris" : ["http://www.mendeley.com/documents/?uuid=9a086d30-fe25-4b0f-8a3c-4a9178d84429"] }], "mendeley" : { "formattedCitation" : "(Berendsen et al., 1984; Bussi et al., 2007)", "plainTextFormattedCitation" : "(Berendsen et al., 1984; Bussi et al., 2007)", "previouslyFormattedCitation" : "(Berendsen et al., 1984; Bussi et al., 2007)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}**. Employing a virtual site model for hydrogen atoms **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1002/(SICI)1096-987X(199906)20:8<786::AID-JCC5>3.0.CO;2-B", "ISBN" : "0192-8651", "ISSN" : "1096-987X", "abstract" : "A systematic analysis is performed on the e\ufb00ectiveness of removing degrees of freedom from hydrogen atoms and/or increasing hydrogen masses to increase the e\ufb00iciency of molecular dynamics simulations of hydrogen-rich systems such as proteins in water. In proteins, high-frequency bond-angle vibrations involving hydrogen atoms limit the time step to 3 fs, which is already a factor of 1.5 beyond the commonly used time step of 2 fs. Removing these degrees of freedom from the system by constructing hydrogen atoms as dummy atoms, allows the time step to be increased to 7 fs, a factor of 3.5 compared with 2 fs. Additionally, a gain in simulation stability can be achieved by increasing the masses of hydrogen atoms with remaining degrees of freedom from 1 to 4 u. Increasing hydrogen mass without removing the high-frequency degrees of freedom allows the time step to be increased only to 4 fs, a factor of two, compared with 2 fs. The net gain in e\ufb00iciency of sampling con\ufb01gurational space may be up to 15% lower than expected from the increase in time step due to the increase in viscosity and decrease in di\ufb00usion constant. In principle, introducing dummy atoms and increasing hydrogen mass do not in\ufb02uence

thermodynamical properties of the system and dynamical properties are shown to be in\ufb02uenced only to a moderate degree. Comparing the maximum time step attainable with these methods (7 fs) to the time step of 2 fs that is routinely used in simulation, and taking into account the increase in viscosity and decrease in di\ufb00usion constant, we can say that a net gain in simulation e\ufb00iciency of a factor of 3 to 3.5 can be achieved.\ufffd\ufffd\ufffd\ufffd1999 John Wiley & Sons, Inc.\ufffd\ufffd\ufffdJ Comput Chem 20: 786-798, 1999", "author" : [{ "dropping-particle" : "", "family" : "Feenstra", "given" : "K. Anton", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hess", "given" : "Berk", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Berendsen", "given" : "Herman J. C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Computational Chemistry", "id" : "ITEM-1", "issue" : "8", "issued" : { "date-parts" : [["1999"]] }, "page" : "786\u2015798", "title" : "Improving efficiency of large time-scale molecular dynamics simulations of hydrogen-rich systems", "type" : "article-journal", "volume" : "20" }, "uris" : ["http://www.mendeley.com/documents/?uuid=61faf993-f403-47af-ad29-94d6b03cc373"] }], "mendeley" : { "formattedCitation" : "(Feenstra et al., 1999)", "plainTextFormattedCitation" : "(Feenstra et al., 1999)", "previouslyFormattedCitation" : "(Feenstra et al., 1999)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** allowed the use of 4-fs time steps during the simulation. All simulations were performed with the GROMACS software package, version 5.1.2 **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.softx.2015.06.001", "ISBN" : "23527110", "ISSN" : "23527110", "abstract" : "GROMACS is one of the most widely used open-source and free software codes in chemistry, used primarily for dynamical simulations of biomolecules. It provides a rich set of calculation types, preparation and analysis tools. Several advanced techniques for free-energy calculations are supported. In version 5, it reaches new performance heights, through several new and enhanced parallelization algorithms. These work on every level; SIMD registers inside cores, multithreading, heterogeneous CPU-GPU acceleration, state-of-the-art 3D domain decomposition, and ensemble-level parallelization through built-in replica exchange and the separate Copernicus framework. The latest best-in-class compressed trajectory storage format is supported.", "author" : [{ "dropping-particle" : "", "family" : "Abraham", "given" : "Mark James", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "",

"family" : "Murtola", "given" : "Teemu", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Schulz", "given" : "Roland", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "P\u00e1ll", "given" : "Szil\u00e1rd", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Smith", "given" : "Jeremy C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Hess", "given" : "Berk", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Lindahl", "given" : "Erik", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "SoftwareX", "id" : "ITEM-1", "issued" : { "date-parts" : [["2015", "9"]] }, "page" : "19-25", "title" : "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers", "type" : "article-journal", "volume" : "1-2" }, "uris" : ["http://www.mendeley.com/documents/?uuid=4366eba6-0771-4147-8a18-0d5c05ac0ce2"] }], "mendeley" : { "formattedCitation" : "(Abraham et al., 2015)", "plainTextFormattedCitation" : "(Abraham et al., 2015)", "previouslyFormattedCitation" : "(Abraham et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

pK_a calculations

The pK_a calculations were performed using a continuum electrostatics method, namely the Poisson-Boltzmann/Monte Carlo (PB/MC) approach, on multiple snapshots taken at a 2 ns interval from different umbrella sampling simulations in the carbachol-bound active state (both for Y440$^{7.53}$ in the upward and downward conformations) and in the apo active state. PB calculations were performed using MEAD (version 2.2.9)**{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/0022-2836(92)91009-E", "ISBN" : "0022-2836 (Print)\\r0022-2836 (Linking)", "ISSN" : "00222836", "PMID" : "1313886", "abstract" : "The effects of solvation and charge-charge interactions on the pKa of ionizable groups in bacteriorhodopsin have been studied using a macroscopic dielectric model with atom-level detail. The calculations are based on the atomic model for bacteriorhodopsin recently proposed by Henderson et al. Even if the structural data are not resolved at the atomic level, such calculations can indicate the quality of the model, outline some general aspects of electrostatic interactions in membrane proteins, and predict some features. The effects of structural uncertainties on the calculations have been investigated by conformational sampling. The results are in

reasonable agreement with experimental measurements of several unusually large pKa shifts (e.g. the experimental findings that Asp96 and Asp115 are protonated in the ground state over a wide pH range). In general, we find that the large unfavorable desolvation energies of forming charges in the protein interior must be compensated by strong favorable charge-charge interactions, with the result that the titrations of many ionizable groups are strongly coupled to each other. We find several instances of complex titration behavior due to strong electrostatic interactions between titrating sites, and suggest that such behavior may be common in proton transfer systems. We also propose that they can help to resolve structural ambiguities in the currently available density map. In particular, we find better agreement between theory and experiment when a structural ambiguity in the position of the Arg82 side-chain is resolved in favor of a position near the Schiff base.", "author" : [{ "dropping-particle" : "", "family" : "Bashford", "given" : "Donald", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gerwert", "given" : "Klaus", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal of Molecular Biology", "id" : "ITEM-1", "issue" : "2", "issued" : { "date-parts" : [["1992", "3"]] }, "page" : "473-486", "title" : "Electrostatic calculations of the pKa values of ionizable groups in bacteriorhodopsin", "type" : "article-journal", "volume" : "224" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a539a42a-edd8-4e9e-ad97-0c4fe44c94e3"] }], "mendeley" : { "formattedCitation" : "(Bashford and Gerwert, 1992)", "plainTextFormattedCitation" : "(Bashford and Gerwert, 1992)", "previouslyFormattedCitation" : "(Bashford and Gerwert, 1992)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}** with a dielectric constant (ε_p) of 4 for the protein and 80 for the solvent (ε_w), in the presence of an explicit membrane. Both $D69^{2.50}$ and $D103^{3.32}$ were included as titrating sites, together with $Y440^{7.53}$ and other nearby tyrosine residues. The temperature was set to 310 K and the ionic strength to 0.145 M. The same temperature was used for MC calculations (10^3 steps in each calculation), which were performed using MCRP **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/S0006-3495(99)77452-7", "ISBN" : "3511446961", "ISSN" : "00063495", "PMID" : "10354425", "abstract" : "A new method is presented for simulating the simultaneous binding equilibrium of electrons and protons on protein molecules, which makes it possible to study the full equilibrium thermodynamics of redox and protonation processes, including electron-proton coupling. The simulations using this method reflect directly the pH and electrostatic potential of the environment, thus providing

a much closer and realistic connection with experimental parameters than do usual methods. By ignoring the full binding equilibrium, calculations usually overlook the twofold effect that binding fluctuations have on the behavior of redox proteins: first, they affect the energy of the system by creating partially occupied sites; second, they affect its entropy by introducing an additional empty/occupied site disorder (here named occupational entropy). The proposed method is applied to cytochrome c3 of Desulfovibrio vulgaris Hildenborough to study its redox properties and electron-proton coupling (redox-Bohr effect), using a continuum electrostatic method based on the linear Poisson-Boltzmann equation. Unlike previous studies using other methods, the full reduction order of the four hemes at physiological pH is successfully predicted. The sites more strongly involved in the redox-Bohr effect are identified by analysis of their titration curves/surfaces and the shifts of their midpoint redox potentials and pKa values. Site-site couplings are analyzed using statistical correlations, a method much more realistic than the usual analysis based on direct interactions. The site found to be more strongly involved in the redox-Bohr effect is propionate D of heme I, in agreement with previous studies; other likely candidates are His67, the N-terminus, and propionate D of heme IV. Even though the present study is limited to equilibrium conditions, the possible role of binding fluctuations in the concerted transfer of protons and electrons under nonequilibrium conditions is also discussed. The occupational entropy contributions to midpoint redox potentials and pKa values are computed and shown to be significant.", "author" : [{ "dropping-particle" : "", "family" : "Baptista", "given" : "Ant\u00f3nio M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Martel", "given" : "Paulo J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Soares", "given" : "Cl\u00e1udio M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical Journal", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["1999", "6"]] }, "page" : "2978-2998", "title" : "Simulation of Electron-Proton Coupling with a Monte Carlo Method: Application to Cytochrome c3 Using Continuum Electrostatics", "type" : "article-journal", "volume" : "76" }, "uris" : ["http://www.mendeley.com/documents/?uuid=68c0a2ac-6ed6-42ea-a352-b1e6303f2b18"] }], "mendeley" : { "formattedCitation" : "(Baptista et al., 1999)", "plainTextFormattedCitation" : "(Baptista et al., 1999)", "previouslyFormattedCitation" : "(Baptista et al., 1999)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** . Each MC step consisted of a cycle of random choices of a state for all individual sites and

pairs of sites with couplings above 2.0 pK_a units **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/S0006-3495(99)77452-7", "ISBN" : "3511446961", "ISSN" : "00063495", "PMID" : "10354425", "abstract" : "A new method is presented for simulating the simultaneous binding equilibrium of electrons and protons on protein molecules, which makes it possible to study the full equilibrium thermodynamics of redox and protonation processes, including electron-proton coupling. The simulations using this method reflect directly the pH and electrostatic potential of the environment, thus providing a much closer and realistic connection with experimental parameters than do usual methods. By ignoring the full binding equilibrium, calculations usually overlook the twofold effect that binding fluctuations have on the behavior of redox proteins: first, they affect the energy of the system by creating partially occupied sites; second, they affect its entropy by introducing an additional empty/occupied site disorder (here named occupational entropy). The proposed method is applied to cytochrome c3 of Desulfovibrio vulgaris Hildenborough to study its redox properties and electron-proton coupling (redox-Bohr effect), using a continuum electrostatic method based on the linear Poisson-Boltzmann equation. Unlike previous studies using other methods, the full reduction order of the four hemes at physiological pH is successfully predicted. The sites more strongly involved in the redox-Bohr effect are identified by analysis of their titration curves/surfaces and the shifts of their midpoint redox potentials and pKa values. Site-site couplings are analyzed using statistical correlations, a method much more realistic than the usual analysis based on direct interactions. The site found to be more strongly involved in the redox-Bohr effect is propionate D of heme I, in agreement with previous studies; other likely candidates are His67, the N-terminus, and propionate D of heme IV. Even though the present study is limited to equilibrium conditions, the possible role of binding fluctuations in the concerted transfer of protons and electrons under nonequilibrium conditions is also discussed. The occupational entropy contributions to midpoint redox potentials and pKa values are computed and shown to be significant.", "author" : [{ "dropping-particle" : "", "family" : "Baptista", "given" : "Ant\u00f3nio M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Martel", "given" : "Paulo J", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Soares", "given" : "Cl\u00e1udio M", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Biophysical Journal", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["1999", "6"]] }, "page" : "2978-2998", "title" : "Simulation of Electron-Proton Coupling with a

Monte Carlo Method: Application to Cytochrome c3 Using Continuum Electrostatics", "type" : "article-journal", "volume" : "76" }, "uris" : ["http://www.mendeley.com/documents/?uuid=68c0a2ac-6ed6-42ea-a352-b1e6303f2b18"] }], "mendeley" : { "formattedCitation" : "(Baptista et al., 1999)", "plainTextFormattedCitation" : "(Baptista et al., 1999)", "previouslyFormattedCitation" : "(Baptista et al., 1999)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}**, whose acceptance/rejection followed a Metropolis criterion **{** ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "http://dx.doi.org/10.1063/1.1699114", "ISBN" : "doi:10.1063/1.1699114", "ISSN" : "00219606", "PMID" : "2797", "abstract" : "A general method, suitable for fast computing machines, for investigating such properties as equations of state for substances consisting of interacting individual molecules is described. The method consists of a modified Monte Carlo integration over configuration space. Results for the two\u2010dimensional rigid\u2010sphere system have been obtained on the Los Alamos MANIAC and are presented here. These results are compared to the free volume equation of state and to a four\u2010term virial coefficient expansion.", "author" : [{ "dropping-particle" : "", "family" : "Metropolis", "given" : "Nicholas", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosenbluth", "given" : "Arianna W.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Rosenbluth", "given" : "Marshall N.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Teller", "given" : "Augusta H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Teller", "given" : "Edward", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Journal Chemical Physics", "id" : "ITEM-1", "issue" : "6", "issued" : { "date-parts" : [["1953"]] }, "page" : "1087-1092", "title" : "Equation of state calculations by fast computing machines", "type" : "article", "volume" : "21" }, "uris" : ["http://www.mendeley.com/documents/?uuid=5ce2844c-8442-4cdc-9907-38fd86711613"] }], "mendeley" : { "formattedCitation" : "(Metropolis et al., 1953)", "plainTextFormattedCitation" : "(Metropolis et al., 1953)", "previouslyFormattedCitation" : "(Metropolis et al., 1953)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" } **}** ; tautomeric forms were not included.

Residue conservation

The GROMACS software package, version 5.0.4 analysis toolkit was used to identify residues with non-hydrogen heavy atoms within 4 Å of the sodium ion path during the simulations. The residue conservation profile of the amino acids was obtained from the GPCRdb server **{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.tips.2014.11.001", "ISBN" : "1873-3735 (Electronic)\\r0165-6147 (Linking)", "ISSN" : "01656147", "PMID" : "25541108", "abstract" : "Generic residue numbers facilitate comparisons of, for example, mutational effects, ligand interactions, and structural motifs. The numbering scheme by Ballesteros and Weinstein for residues within the class A GPCRs (G protein-coupled receptors) has more than 1100 citations, and the recent crystal structures for classes B, C, and F now call for a community consensus in residue numbering within and across these classes. Furthermore, the structural era has uncovered helix bulges and constrictions that offset the generic residue numbers. The use of generic residue numbers depends on convenient access by pharmacologists, chemists, and structural biologists. We review the generic residue numbering schemes for each GPCR class, as well as a complementary structure-based scheme, and provide illustrative examples and GPCR database (GPCRDB) web tools to number any receptor sequence or structure.", "author" : [{ "dropping-particle" : "", "family" : "Isberg", "given" : "Vignir", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Graaf", "given" : "Chris", "non-dropping-particle" : "de", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Bortolato", "given" : "Andrea", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cherezov", "given" : "Vadim", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Katritch", "given" : "Vsevolod", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Marshall", "given" : "Fiona H.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Mordalski", "given" : "Stefan", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Pin", "given" : "Jean-Philippe", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Stevens", "given" : "Raymond C.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Vriend", "given" : "Gerrit", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "",

"family" : "Gloriam", "given" : "David E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "container-title" : "Trends in Pharmacological Sciences", "id" : "ITEM-1", "issue" : "1", "issued" : { "date-parts" : [["2015", "1"]] }, "page" : "22-31", "publisher" : "Elsevier Ltd", "title" : "Generic GPCR residue numbers \u2013 aligning topology maps while minding the gaps", "type" : "article-journal", "volume" : "36" }, "uris" : ["http://www.mendeley.com/documents/?uuid=069e307d-2306-4fdf-af5b-404cb9d34de4"] }], "mendeley" : { "formattedCitation" : "(Isberg et al., 2015)", "plainTextFormattedCitation" : "(Isberg et al., 2015)", "previouslyFormattedCitation" : "(Isberg et al., 2015)" }, "properties" : { "noteIndex" : 0 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**.

QUANTIFICATION AND STATISTICAL ANALYSIS

The standard deviation of the PMF profiles was estimated by using the Bayesian bootstrap method, as implemented in gmx wham, with 200 runs. Errors for the gating charge calculation were estimated by deriving the minimum and maximum slopes of the charge imbalance-voltage relationships, giving a mean and standard deviation for the capacitance of the membrane-patch system. This error was then propagated into the determination of the gating charges.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
N/A	N/A	N/A
Bacterial and Virus Strains		
N/A	N/A	N/A
Biological Samples		
N/A	N/A	N/A
Chemicals, Peptides, and Recombinant Proteins		
N/A	N/A	N/A
Critical Commercial Assays		
N/A	N/A	N/A
Deposited Data		

N/A	N/A	N/A
Experimental Models: Cell Lines		
N/A	N/A	N/A
Experimental Models: Organisms/Strains		
N/A	N/A	N/A
Oligonucleotides		
N/A	N/A	N/A
Recombinant DNA		
N/A	N/A	N/A
Software and Algorithms		

GROMACS	{ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/j.softx.2015.06.001", "ISBN" : "23527110", "ISSN" : "23527110", "abstract" : "GROMACS is one of the most widely used open-source and free software codes in chemistry, used primarily for dynamical simulations of biomolecules. It provides a rich set of calculation types, preparation and analysis tools. Several advanced techniques for free-energy calculations are supported. In version 5, it reaches new performance heights, through several new and enhanced parallelization algorithms. These work on every level; SIMD registers inside cores, multithreading, heterogeneous CPU-GPU acceleration, state-of-the-art 3D domain decomposition, and ensemble-level parallelization through built-in replica exchange and the separate Copernicus framework. The latest best-in-class compressed trajectory storage format is supported.", "author" : [{ "dropping-particle" : "", "family" : "Abraham", "given" : "Mark James", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Murtola", "given" : "Teemu", "non-dropping-particle" : "",	http://www.gromacs.org/
{PAGE }		

MEAD {PAGE }	{ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/0022-2836(92)91009-E", "ISBN" : "0022-2836 (Print)\\r0022-2836 (Linking)", "ISSN" : "00222836", "PMID" : "1313886", "abstract" : "The effects of solvation and charge-charge interactions on the pKa of ionizable groups in bacteriorhodopsin have been studied using a macroscopic dielectric model with atom-level detail. The calculations are based on the atomic model for bacteriorhodopsin recently proposed by Henderson et al. Even if the structural data are not resolved at the atomic level, such calculations can indicate the quality of the model, outline some general aspects of electrostatic interactions in membrane proteins, and predict some features. The effects of structural uncertainties on the calculations have been investigated by conformational sampling. The results are in reasonable agreement with experimental measurements of several unusually large pKa shifts (e.g. the experimental findings that Asp96 and Asp115 are protonated in the ground state over a wide pH range). In general, we find that the large unfavorable desolvation energies of forming charges in the protein interior	http://www.bisb.uni-bayreuth.de/People/ullmannt/

AMBER16	{ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Case", "given" : "D", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Betz", "given" : "RM", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Botello-Smith", "given" : "W.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cerutti", "given" : "D.S.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Cheatham", "given" : "T.E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Darden", "given" : "T.A.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Duke", "given" : "R.E.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Giese", "given" : "T.J.", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Gohlke", "given" :	http://ambermd.org/
{PAGE }		

GOLD	**{**ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "author" : [{ "dropping-particle" : "", "family" : "Willett", "given" : "Peter", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }, { "dropping-particle" : "", "family" : "Glen", "given" : "C", "non-dropping-particle" : "", "parse-names" : false, "suffix" : "" }], "id" : "ITEM-1", "issued" : { "date-parts" : [["1995"]] }, "page" : "43-53", "title" : "Molecular Recognition of Receptor Sites using a Genetic Algorithm with a Description of Desolvation", "type" : "article-journal" }, "uris" : ["http://www.mendeley.com/documents/?uuid=a8114da6-d70a-44fe-810a-f773eceb56ec"] }], "mendeley" : { "formattedCitation" : "(Willett and Glen, 1995)", "plainTextFormattedCitation" : "(Willett and Glen, 1995)", "previouslyFormattedCitation" : "(Willett and Glen, 1995)" }, "properties" : { "noteIndex" : 25 }, "schema" : "https://github.com/citation-style-language/schema/raw/master/csl-citation.json" }**}**	https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/

MCRP	{ADDIN CSL_CITATION { "citationItems" : [{ "id" : "ITEM-1", "itemData" : { "DOI" : "10.1016/S0006-3495(99)77452-7", "ISBN" : "3511446961", "ISSN" : "00063495", "PMID" : "10354425", "abstract" : "A new method is presented for simulating the simultaneous binding equilibrium of electrons and protons on protein molecules, which makes it possible to study the full equilibrium thermodynamics of redox and protonation processes, including electron-proton coupling. The simulations using this method reflect directly the pH and electrostatic potential of the environment, thus providing a much closer and realistic connection with experimental parameters than do usual methods. By ignoring the full binding equilibrium, calculations usually overlook the twofold effect that binding fluctuations have on the behavior of redox proteins: first, they affect the energy of the system by creating partially occupied sites; second, they affect its entropy by introducing an additional empty/occupied site disorder (here named occupational entropy). The proposed method is applied to cytochrome c3 of Desulfovibrio vulgaris Hildenborough to study its redox	http://www.itqb.unl.pt/labs/molecular-simulation
{PAGE }		

Other		
N/A	N/A	N/A

Intracellular Transfer of Na^+ in an Active State G-Protein Coupled Receptor

SUPPLEMENTAL INFORMATION

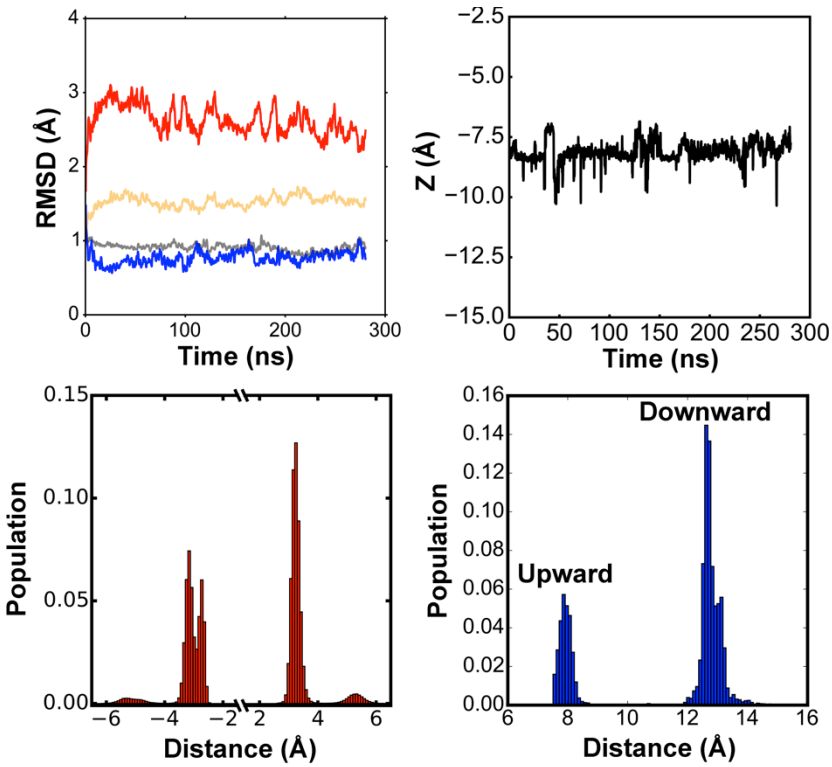

Figure S1 – related to Figure 1: System preparation.

(Top left) The red and blue lines correspond to the RMSD of helix TM6 relative to the inactive and active state structures (PDB: 3UON, 4MQT), respectively. The grey and orange lines correspond to the RMSD of all transmembrane helices relative to the inactive and active state structures, respectively. The RMSD of TM6 with respect to the active state structure remains below 1 Å following the transition to the active state (blue line: TM6 vs. active). The RMSDs of all seven TM helices were calculated using the backbone atoms of residues 25-46, 60-83, 96-123, 140-158, 189-210, 388-408 and 422-443; those of TM6 - from residues 388-408. **(Top right)** Position of Na^+ during a targeted MD simulation from the inactive to the active state of m2r. The position of the Na^+ ion remains stable throughout the transition of the m2r from the inactive to active conformation. The position is reported relative to the $D103^{3.32}$ Cα atom in the orthosteric ligand binding pocket. **(Bottom left)** Distance distribution between Na^+ and $D69^{2.50}$ (Cγ) in the active state of m2r under -250 mV and charged $D69^{2.50}$. Positive values signify displacements towards the extracellular side, negative values movements towards the intracellular side. **(Bottom right)** Distance distribution between the atoms $Y440^{7.53}$ (OH) and $D69^{2.50}$ (Cα) under a V_m of ~250 mV, with a protonated $D69^{2.50}$, displaying the two most populated states of $Y440^{7.53}$: an upward (~8 Å) and downward (~12.5 Å) conformation.

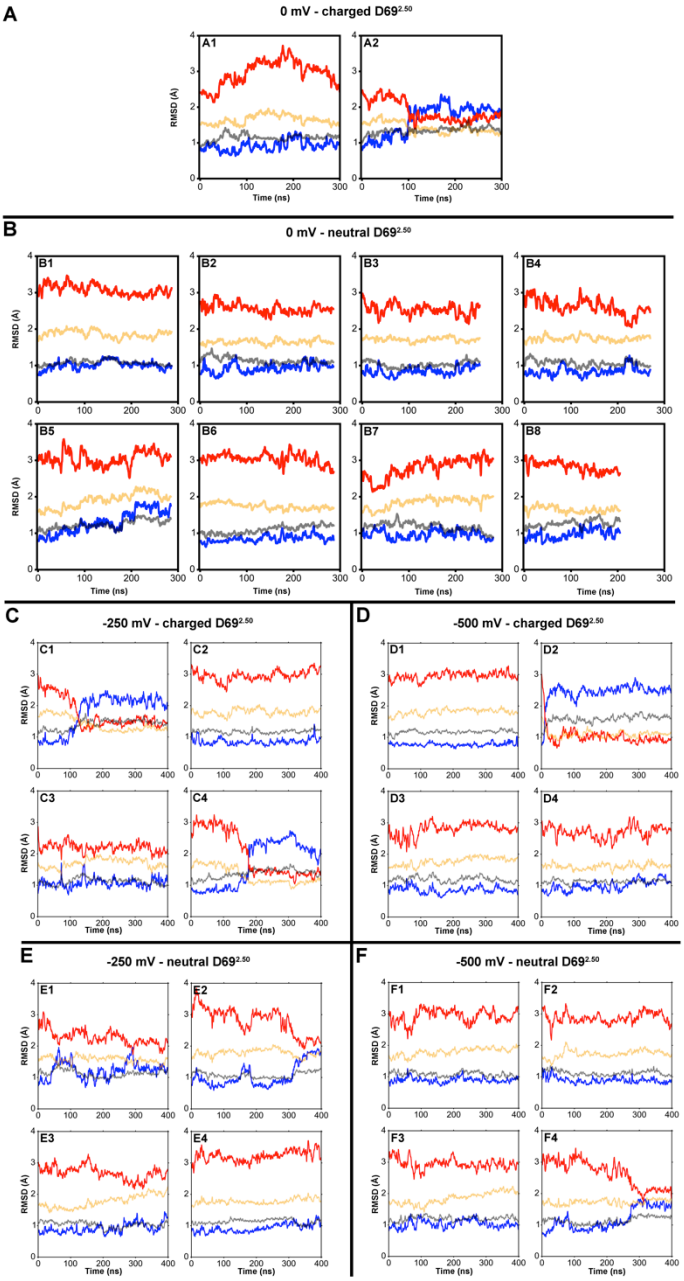

Figure S2 – related to Figure 3: Backbone RMSD values during MD simulations of the active state M2R under equilibrium (A, B) and negative V_m (C-F).

The red and blue lines show RMSD values of helix TM6 with respect to the inactive and active state structures (PDB: 3UON, 4MQT), respectively, while the grey and orange lines show the RMSDs of all TM helices relative to the inactive and active state structures. $D69^{2.50}$ was either charged (**A**, **C** and **D**) or neutral (**B**, **E** and **F**). Also see Fig 3 and corresponding discussion in the main text. When the Na^+ ion is still bound to $D69^{2.50}$ in simulations with a charged $D69^{2.50}$, the receptor can switch back to an inactive conformation (e.g. panels **C1**, **C4** and **D2**; compare grey and orange lines). In contrast, most simulations with a neutral $D69^{2.50}$ remain stable in the active state and only two simulations show a change to an intermediate conformation (panels **E1** and **F4**), but no further transitions reverting to the inactive state.

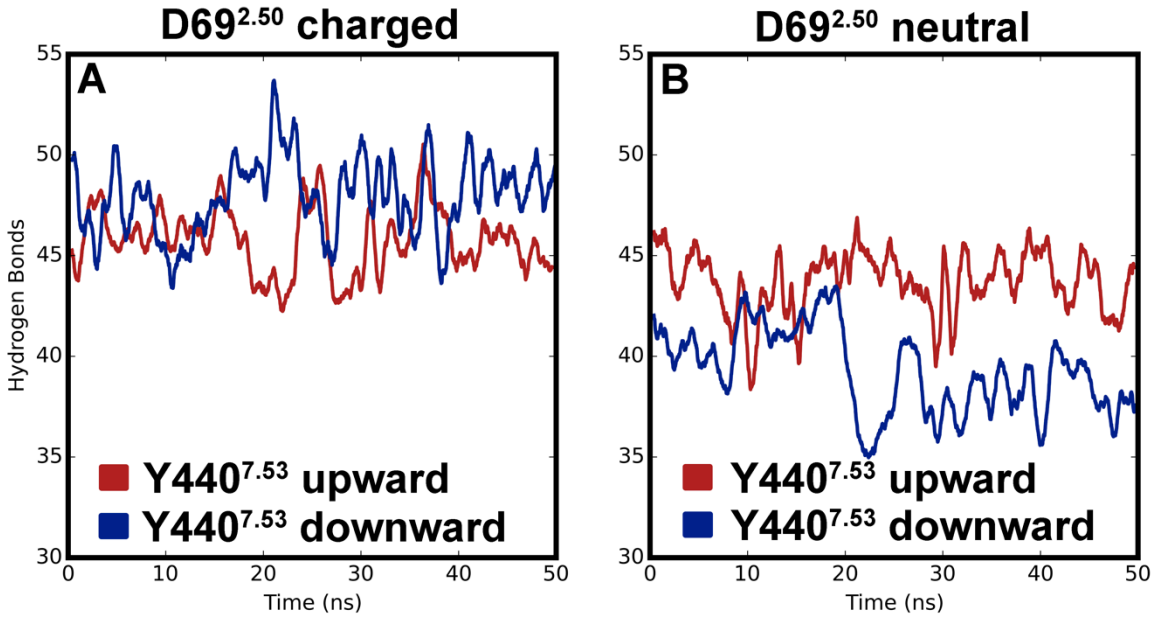

Figure S3 – related to Figure 4: Number of hydrogen bonds around the Na^+ binding site.

The conformation of the $Y440^{7.53}$ sidechain and the protonation state of $D^{2.50}$ affect the number of hydrogen bonds in the hydrophilic region around the Na^+ binding site, defined as lying within 10 Å of the C_γ atom of $D69^{2.50}$. **(A)** With a charged $D69^{2.50}$, a similar number of hydrogen bonds exist, irrespective of the conformation of $Y440^{7.53}$. **(B)** In the case of neutral $D^{2.50}$, the downward conformation of $Y440^{7.53}$ leads to a decrease in the number of hydrogen bonds around the Na^+ binding pocket.

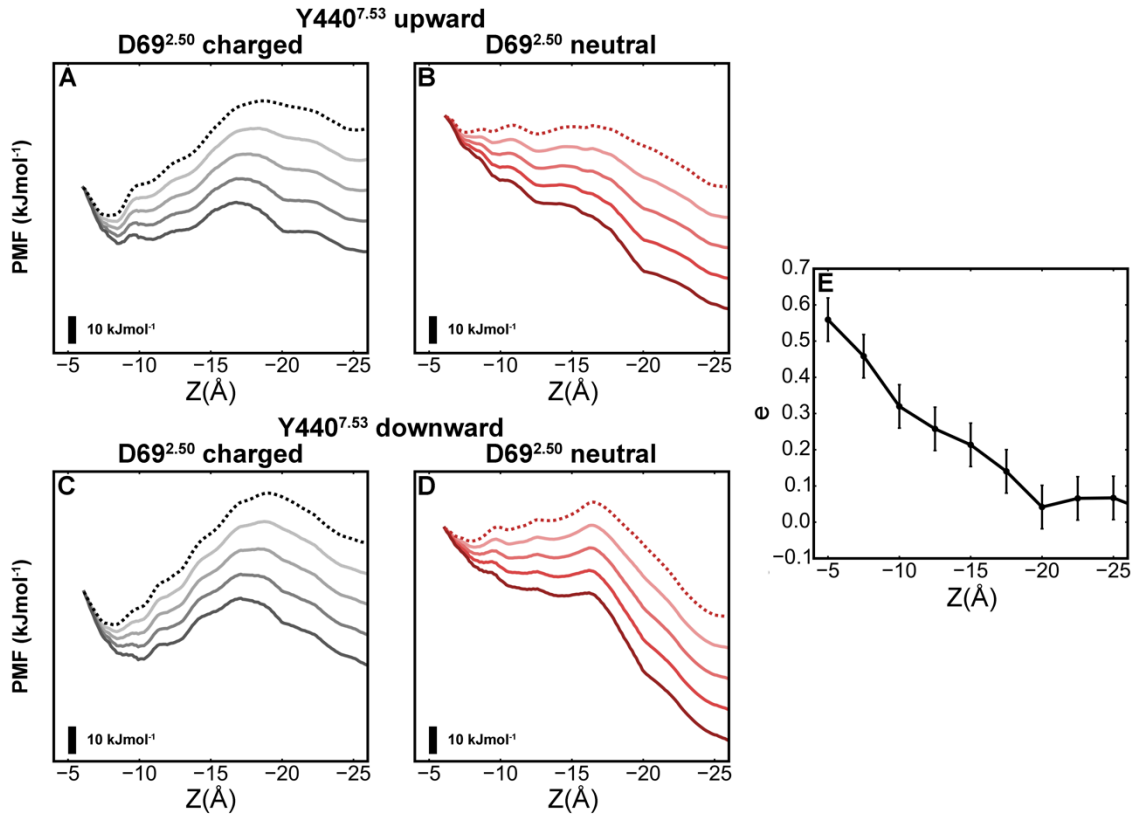

Figure S4 – related to Figure 4: Non-equilibrium effect of V_m upon the PMF profiles of Na^+ translocation to the cytoplasm

(**A**-**D**) Voltage-induced tilt of the free energy profiles (see Fig 4 in the main text) of Na^+ translocation in m2r in a non-equilibrium case under 4 different conditions: (**A**) $Y440^{7.53}$ upward and $D69^{2.50}$ charged, (**B**) $Y440^{7.53}$ upward and $D69^{2.50}$ neutral, (**C**) $Y440^{7.53}$ downward and $D69^{2.50}$ charged, and (**D**) $Y440^{7.53}$ downward and $D69^{2.50}$ neutral. Increments are from 250 mV (light lines) to 1000 mV (dark lines); a dotted line indicates 0 mV. Similar to the figures in the main text, the Z-coordinate along the vertical axis was calculated relative to the Cα atom of $D103^{3.32}$. The underlying voltage drop was mapped using the calculated gating charge (see below). The panels display the relative free energy differences for each voltage regime; the black bar denotes an energy difference of 10 kJ mol^{-1} within each panel. (**E**) The gating charge, q, was calculated from the simulation of m2r with $Y440^{7.53}$ upward and $D69^{2.50}$ charged (see the Methods section in the main text for details) and is shown in units of the elementary charge *e*.

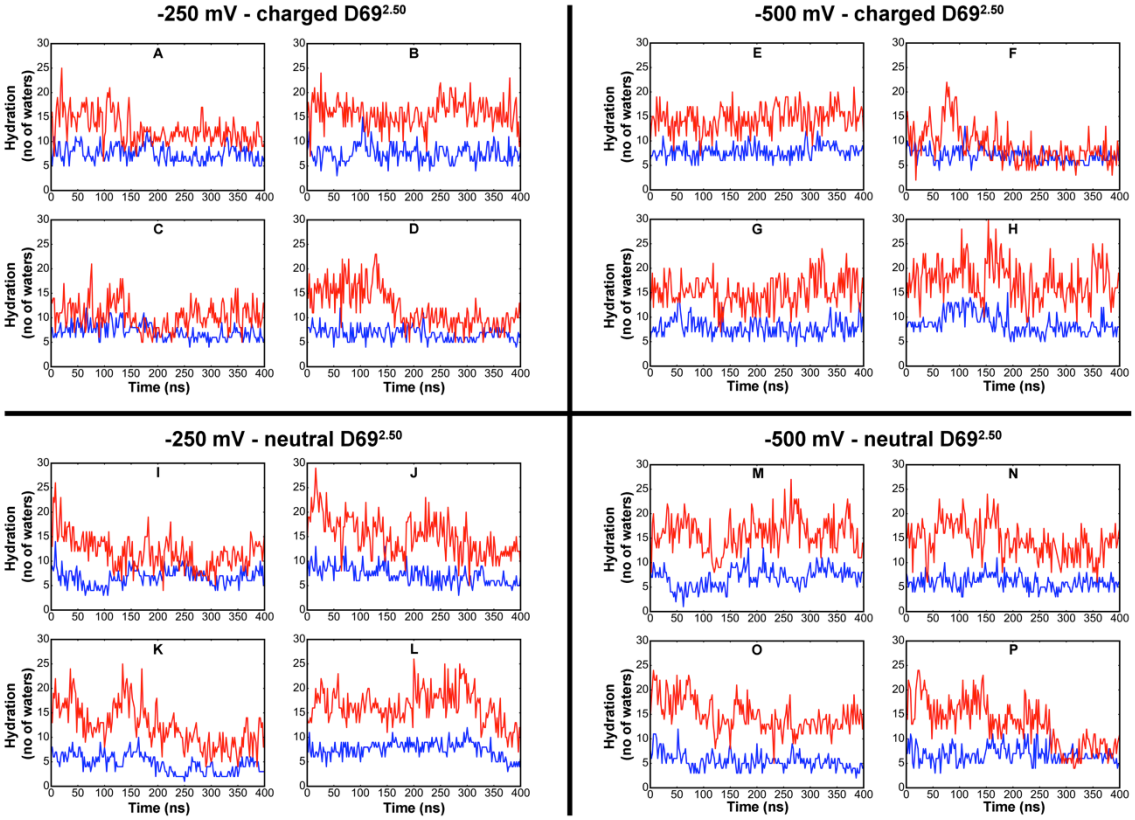

Figure S5 – related to Figure 6: Hydration of the hydrophilic pocket and intracellular effector-binding site.

The hydration level of the m2r receptor is represented by a number of water molecules within two regions: the hydrophilic pocket (blue lines) and the intracellular effector-binding site (red lines). Here we show 4 replicates of each simulation condition: (**A-D**) V_m -250 mV, $D69^{2.50}$ charged, (**E-H**) V_m -500 mV, $D69^{2.50}$ charged, (**I-L**) V_m -250 mV, $D69^{2.50}$ neutral, and (**M-P**) V_m -500 mV, $D69^{2.50}$ neutral. The hydrophilic pocket was defined as Z = ± 4 Å from the $D69^{2.50}$ Cα atom. The channel to the effector-binding site was defined between the Z = -4 Å from the $D69^{2.50}$ Cα atom and the Z-coordinate of the $R121^{3.50}$ Cα. The number of water molecules was recorded every 2 ns throughout the simulations. Also see Table S1 for the mean values.

It can be seen that the number of water molecules within the hydrophilic pocket remains stable throughout all simulations, with a slight decrease when $D69^{2.50}$ is neutral (panels I-P and Table S1). The number of water molecules within the intracellular effector-binding site is stable in the majority of simulations, with a substantial decrease in few cases correlating with the transition of those systems to the inactive or intermediate states (Fig S5 and Table S1).

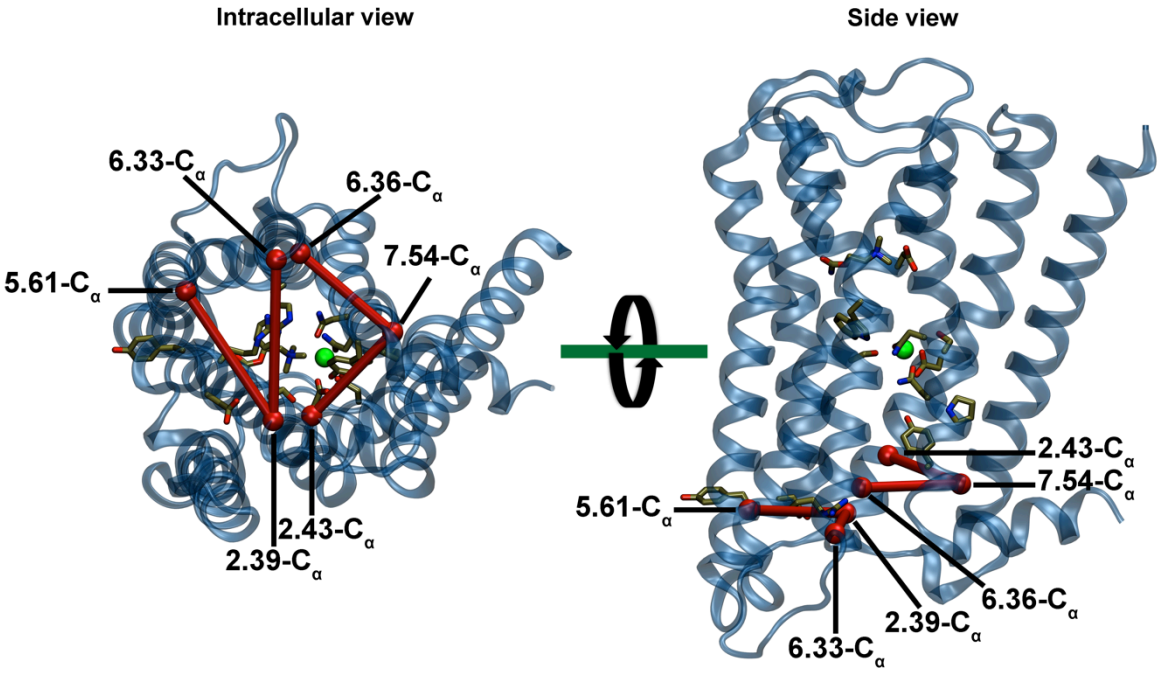

Figure S6 – related to Figure 1: Depiction of the minimal set of distance restraints used to maintain the active conformation of the m2r at the G-protein binding site.

A set of four distance restraints (indicated by red bars) was applied to the intracellular portion of the transmembrane helices as described in the Methods section. This procedure serves to maintain the m2r in the active state despite the absence of a bound G-protein. With the Na^+ ion bound at $D69^{2.50}$, and $D69^{2.50}$ in a charged state, in many simulations without these restraints the receptor regains its inactive conformation on timescales below microseconds, which is in agreement with the role of sodium as stabilizer of the inactive conformation.

$D^{2.50}$ charge state	Transmembrane potential (mV)	Replicate	Na^+ permeation	Hydrophilic pocket hydration	Effector pocket hydration
Negatively charged (deprotonated)	-250	1	No	7.6±1.6	12.6±3.1
		2	No	7.5±1.6	15.8±2.7
		3	No	7.2±1.6	10.4±2.9
		4	No	6.7±1.3	12.1±4.1
Negatively charged (deprotonated)	-500	1	No	7.9±1.4	14.6±2.6
		2	No	7.0±1.4	9.1±3.7
		3	**Yes**	7.9±1.8	15.2±3.1
		4	No	8.7±2.1	17.5±4.0
Neutral (protonated)	-250	1	**Yes**	6.7±1.7	11.8±3.4
		2	**Yes**	7.2±1.8	15.1±4.0
		3	**Yes**	4.7±1.9	12.2±4.3
		4	No	7.6±1.6	16.4±3.5
Neutral (protonated)	-500	1	**Yes**	6.9±2.0	15.9±3.5
		2	**Yes**	6.0±1.5	14.5±3.5
		3	**Yes**	5.5±1.7	14.8±3.3
		4	**Yes**	6.8±1.7	13.0±4.7

Table S1 – related to figure 3: Permeation of Na^+ into the cytoplasm and hydration of the m2r receptor.

A list of simulations performed in the presence of V_m detailing the simulation conditions and main observations: TM potential, the charge state of $D69^{2.50}$, observation of Na^+ permeation to the intracellular side, and hydration of the receptor – the number of water molecules (mean ± std) in the hydrophilic pocket (Z = ±4 Å from the $D69^{2.50}$ Cα atom) and in the hydrated channel (between the Z = -4 Å from the $D69^{2.50}$ Cα atom and the Z-coordinate of the $R121^{3.50}$ Cα). For each condition four independent replicates were simulated for 400 ns. The number of water molecules was recorded every 2 ns throughout the simulations. Also see Fig S9.

Residue	Consensus among muscarinic receptors	Consensus among aminergic receptors	Consensus among class A GPCRs
N41$^{1.50}$	N (100%)	N (100%)	N (98%)
N58$^{2.39}$	N (100%)	T (47%), Polar (81%)	T (37%), Polar (54%)
N59$^{2.40}$	N (100%)	N (92%)	N (39%), Polar (73%)
L62$^{2.43}$	L (100%)	I (50%), Hydrophobic (100%)	L (36%), Hydrophobic (96%)
L65$^{2.46}$	L (100%)	L (94%)	L (90%)
A66$^{2.47}$	A (100%)	A (94%)	A (73%)
A68$^{2.49}$	A (100%)	A (64%)	A (56%)
D69$^{2.50}$	D (100%)	D (100%)	D (92%)
I72$^{2.53}$	I (100%)	V (58%), Hydrophobic (100%)	V (23%), Hydrophobic (88%)
V106$^{3.35}$	A (60%), V (40%)	C (44%), Hydrophobic (100%)	N (26%), Hydrophobic (39%)
S107$^{3.36}$	S (100%)	C (56%)	M (19%)
A109$^{3.38}$	A (100%)	A (81%)	A (38%)
S110$^{3.39}$	S (100%)	S (100%)	S (72%)
V111$^{3.40}$	V (100%)	I (81%), Hydrophobic (100%)	I (38%), Hydrophobic (89%)
L114$^{3.43}$	L (100%)	L (89%)	L (73%)
I117$^{3.46}$	I (100%)	I (94%)	I (56%)
D120$^{3.49}$	D (100%)	D (100%)	D (64%)
R121$^{3.50}$	R (100%)	R (100%)	R (94%)
C124$^{3.53}$	S (60%), C (40%)	A (56%), Hydrophobic (64%)	A (47%), Hydrophobic (68%)
Y206$^{5.58}$	Y (100%)	Y (94%)	Y (73%)
V385$^{6.33}$	A (60%), V (40%)	A (72%), Hydrophobic (97%)	A (29%)
T388$^{6.36}$	T (100%)	T (58%)	T (25%)
I392$^{6.40}$	I (100%)	I (53%), Hydrophobic (100%)	V (47%), Hydrophobic (100%)
A395$^{6.43}$	A (100%)	A (39%)	V (31%)
F396$^{6.44}$	F (100%)	F (100%)	F (75%)
T399$^{6.47}$	T (100%)	C (72%)	C (70%)
W400$^{6.48}$	W (100%)	W (100%)	W (68%)
N432$^{7.45}$	N (100%)	N (92%)	N (67%)
S433$^{7.46}$	S (100%)	S (100%)	S (64%)
T434$^{7.47}$	T (100%)	A/T (22%)	C (39%)
I435$^{7.48}$	I (60%), V (40%)	L (33%), Hydrophobic (100%)	L (34%), Hydrophobic (96%)
N436$^{7.49}$	N (100%)	N (100%)	N (71%)
P437$^{7.50}$	P (100%)	P (100%)	P (93%)
C439$^{7.52}$	C (100%)	I (61%), Hydrophobic (100%)	I (41%), Hydrophobic (99%)
Y440$^{7.53}$	Y (100%)	Y (100%)	Y (89%)
C443$^{7.56}$	C (100%)	F (44%)	L (23%)

Table S2 – related to figure 5: Conservation of the pocket and the Na^+ exit channel. Conservation of the residues in the transmembrane region that were observed to be in contact (<4.5 Å) with Na^+ in the MD simulations. Overall, the sodium ion was observed to come into close proximity with 34 residues.