Published in final edited form as:

Curr Opin Struct Biol. 2008 October ; 18(5): 587–592. doi:10.1016/j.sbi.2008.07.005.

Electron crystallography of proteins in membranes

Yoshinori Fujiyoshi¹ and Nigel Unwin²

¹*Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8502, Japan*

²*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK*

Abstract

Electron crystallography has played a vital role in advancing our understanding of proteins in membranes since the 'fluid mosaic model' was proposed in 1972. It is now an established technique to reveal the structures of proteins in their natural bilayer environment and makes possible the study of biological mechanisms through freeze-trapping of transitional states. Thus, images and diffraction patterns of well-ordered, planar and tubular protein-lipid crystals are yielding atomic models, which tell us how the proteins *in situ* are designed and carry out their membrane-specific tasks. Recent methodological advances and the inclusion of tomographic and cryo-sectioning techniques are enabling detailed information to be obtained from increasingly smaller and more disordered membrane assemblies, extending the potential of this approach.

Introduction

The 'fluid mosaic model' of membrane structure, proposed by Singer and Nicholson [1], pictured proteins in membranes as isolated molecules, or complexes, which were similar to proteins in solution, but for the fact that they were integrated within a fluid lipid bilayer. The polypeptide chains were stably incorporated in the bilayer by folding such that their polar and nonpolar parts partitioned according to whether they faced the solvent or the hydrophobic portions of the lipids. While numerous electron and X-ray crystallographic studies of specific membrane proteins have now demonstrated the general validity of this concept, a proper understanding of how any one of them functions may require analysis of its conformation in several different (physiological) settings. In most cases, understanding the precise molecular mechanism of a membrane protein remains a daunting task.

Electron crystallography of protein-lipid arrays (Figure 1) has played a key role in developing our present knowledge of how membrane proteins are designed and work, by elucidating the protein structures in their functional, lipid-associated forms. In 1975, the seven membrane-spanning α -helices of bacteriorhodopsin were resolved, providing the first view of polypeptide chains traversing the lipid bilayer [2]. Later, an atomic model of bacteriorhodopsin was obtained [3]. Currently there have been several other structures solved by this approach [4-7, 8•,9] or determined to resolutions where the α -helical arrangement has become visible (e.g. [10-12,13•]), showing how the polypeptide folds. The ability to examine samples frozen rapidly in a defined solution [14] has contributed greatly to some of these studies, and facilitated experiments to trap physiologically relevant transitional states [15]. Now further methodological advances are being made, allowing increasingly detailed information to be obtained from small and/or disordered membrane assemblies.

Correspondence to: Yoshinori Fujiyoshi; Nigel Unwin.

Corresponding author: Fujiyoshi, Yoshinori (yoshi@em.biophys.kyotou.ac.jp) and Unwin, Nigel (mas@mrc-lmb.cam.ac.uk).

This brief review draws attention to the methodological advances and highlights some of the insights into membrane proteins that have emerged from recent electron crystallographic studies.

Sheets and tubes

Electron crystallographic studies on protein-lipid arrays in the form of sheets (two-dimensional crystals) or tubes (tubular crystals) have given the most informative density maps of membrane proteins, revealing in one case [8•] precise details of individual lipid molecules as well as the protein side chains. Averaging the many unit cells present in each image of a two-dimensional or tubular crystal greatly enhances the poor signal-to-noise ratio owing to the weak electron dose, while taking advantage of the fact the lattice and symmetry elements define accurately the position and orientation of each molecule.

The averaging of isolated membrane proteins to obtain structures is also feasible if they are large enough (>250 kDa), using 'single particle methods', but the alignments are less accurate, making high resolution more difficult to achieve. Also in the absence of lattice contacts (and required presence of detergent), conformational heterogeneity is more likely, introducing additional complications. Nevertheless it is possible in favourable cases to glean information about transmembrane architecture from this kind of specimen [16].

Over the past few years there have been significant advances in methods for producing protein-lipid arrays from detergent-solubilised, purified protein, both in the form of two-dimensional and tubular crystals [17-19]. But extensive robotic screens of the kind developed for three-dimensional crystallisation have not yet been achieved. And still lacking, despite some progress [20], is the ability to check reliably and rapidly the quality of samples produced by the screens. On the contrary, several laboratories have developed the computational methods for analysing images of two-dimensional crystals more efficiently and/or in a more user-friendly way [21-23], extending the value and scope of the original software [24].

Tubular crystals have not yet been used extensively for determining structures, although because of their helical symmetry they offer some significant advantages (and are indeed the natural product of protein crystallising on the surface of a vesicle). For example, one image of a tube contains many different views of the same molecule, enough to reconstruct it roughly in three-dimensions; there is no 'missing cone' of information; and the surfaces of tubes, which are imaged over holes, touch only solvent, avoiding potentially damaging interactions with the carbon support film.

Images of tubes are processed to correct for distortions in a similar way as are those from two-dimensional crystals [3]. Typically, one or two repeat lengths are divided into short segments, each of which is compared with a reference structure to determine the (seven) parameters needed to define completely its three-dimensional alignment [25,26]. The individual aligned segments are then added back to reconstruct the whole repeat. Traditionally, this procedure has been carried out in reciprocal space, using Fourier-Bessel methods [27]. These methods enable critical assessment of the data (e.g. extent of preservation of helical symmetry and of possible twofold symmetry perpendicular to the tube axis) and the ready incorporation of corrections for the focus changes at different levels in the structure [28]. A real-space method has also been developed that avoids the Fourier-Bessel formulation, treating segments as a string of single particles [29•]. This alternative approach is becoming widely used for extracting information from poorly ordered helical polymers, and has also been applied to tubular protein-lipid crystals [30•]. A recent application to a more rigid, filamentous helical assembly ([31••]; Figure 2), demonstrates its potential for determining structures from tubes at near-atomic resolution.

Freeze-trapping different conformational states

The overriding value of electron crystallographic studies of sheets and tubes is that they probe the structures of membrane proteins in their natural lipid environment. Detergent micelles are an imperfect substitution for the lipids encircling the protein *in vivo*, and X-ray structures of membrane proteins in detergents therefore always carry a risk of not representing a biologically relevant state [32,33]. One can further exploit the physiological advantage conferred by the protein-in-lipid specimens, using electron crystallography as a direct means to probe the biological mechanism. A routine step in specimen preparation is plunge-freezing of the electron microscope grid into liquid nitrogen-cooled ethane. This cools the specimen very rapidly ($\sim 10^6$ °C/s; [14]), and so permits efficient trapping of a transient structure that has a life-time of a millisecond or longer. To activate the protein, one can either use light [34], or spray the appropriate ligand onto the grid just before the grid hits the ethane surface [35].

While this technique has not so far been widely used, its initial application to the ACh receptor channel in tubular crystals yielded pivotal insight, allowing a structural model for the gating mechanism to be proposed [6,15]. High quality images of ACh-reacted tubes have been difficult to obtain after the spray-freeze-trapping treatment, but the development of an extremely stable helium-cooled top-entry stage [36] has made the process of data collection much easier, and should eventually permit the gating mechanism to be described at near-atomic resolution.

Molecular tomography

Cryo-electron tomography is a recently developed approach that is providing a new way to explore proteins *in situ*, in their functional context. With this technique, the three-dimensional picture of a whole 'scene' from an ice-embedded specimen is built up by combining information from a large number of tilt views. Although radiation damage imposes a resolution limit of ~ 20 Å, in the absence of any averaging, valuable information about the organisation of a protein can be obtained directly from the tomographic sections. It is then often possible to improve the signal-to-noise ratio by real-space averaging within the three-dimensional volume, taking advantage of symmetry that is present or the fact that the protein forms at least a partly ordered array (e.g. [37-39]). Finally atomic structures, obtained by other means, may be docked into the tomographic densities to show them in their proper functional context.

Reconstituted membranes, isolated membrane fragments and membranes in sections cut from frozen tissue make ideal subjects for application of the real-space averaging and docking procedures. A recent tomographic study of the desmosome in frozen sections [40•] highlights the potential of this approach for exploring the architecture of complex membrane assemblies and organelles.

Special properties of proteins in membranes

The fluid mosaic model did not address the detailed folding of the polypeptide chain within the lipid bilayer, although it predicted the likely predominance of membrane-spanning helices, and the distinct folding of the polypeptide domains exposed to solvent. Some new general properties and principles are beginning to emerge from the combined results of electron and X-ray crystallographic studies. For example, the right-handed helix packing, first seen in aquaporin [41,42], is common in membrane proteins [43], and may confer additional stability in a bilayer environment [44]. Helices in membranes often adopt bent configurations, which enable them to pack more tightly against one another. And the helices may contain a flexible region or hinge point, as in bacteriorhodopsin [45], to enable a rapid localised conformational change.

It is interesting that the loops at the ends of the membrane-spanning helices often form rather tenuous interfaces with the apposed large soluble domains. This makes it likely that many membrane proteins do not in fact undergo rigid quaternary rearrangements typical of soluble allosteric proteins, as envisaged by Singer [46], but instead work as a result of distinct movements in each domain, which are coupled to one another. Ligand-gated ion channels [6, 47,48], and ABC transporters [49] are examples of proteins that seem to act in this way (Figure 3).

Finally ion channels, in controlling ion flow across the membrane, use similar principles as soluble enzymes, which incorporate precise stereo-chemistry to achieve substrate specificity and catalyse a reaction. Thus, the selectivity filter of the potassium channel uses precisely positioned carbonyl groups lining its surface to provide a constricting, yet highly conductive pathway for potassium ions across a part of the bilayer ([50]; Figure 4). The pathway is only about the diameter of a potassium ion (2.7 Å), because the carbonyl groups coordinate with the ion, substituting ideally for the normally tightly bound water molecules. By contrast, the (closed) gate in the ACh receptor makes a much wider opening (about 6 Å diameter at its narrowest point [6]), but is built from a girdle of completely hydrophobic residues. The girdle creates an effective permeation barrier to potassium (and other) ions, because the ion has no opportunity to shed its hydration shell and so is, in effect, too large to go through [6,51].

Conclusions

The ability of electrons to form images, combined with advances in cryo-technology, is enabling us to acquire detailed structural and chemical information about membrane proteins in their physiological lipid and ionic settings. This information complements that obtained by X-ray diffraction of proteins in detergent, where biological relevance of the structure is less certain. Increased computer capacity has encouraged the development of cryo-electron tomography and real-space averaging methods, which are extending the possibilities of obtaining high resolution information from increasingly smaller protein-lipid arrays, and from membrane specialisations *in situ*. Improvements in methods for growing protein-lipid arrays, further development of automatic methods to record and process the images, and ultimately the development of better electron detectors, are keys to extending the future scope and power of this technique.

Acknowledgements

This work was supported partly by an NIH grant RO1GM061941 (NU) and partly by Grants-in-Aid for Specially Promoted Research and NEDO (YF).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Singer SJ, Nicholson GL. The fluid mosaic model of the structure of cell membranes. *Science* 1972;174:720–731. [PubMed: 4333397]
2. Henderson R, Unwin PN. Three-dimensional model of purple membrane obtained by electron microscopy. *Nature* 1975;257:28–32. [PubMed: 1161000]
3. Henderson R, Baldwin JM, Ceska TA, Zemlin F, Beckmann E, Downing KH. Model for the structure of bacteriorhodopsin based on high resolution electron cryo-microscopy. *J Mol Biol* 1990;213:899–929. [PubMed: 2359127]

4. Kuhlbrandt W, Wang DN, Fujiyoshi Y. Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 1994;367:613–621.
5. Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y. Structural determinants of water permeation through aquaporin-1. *Nature* 2000;407:599–605. [PubMed: 11034202]
6. Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* 2003;423:949–955. [PubMed: 12827192]
7. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J Mol Biol* 2005;346:967–989. [PubMed: 15701510]
8. Gonen T, Cheng Y, Sliz P, Hiroaki Y, Fujiyoshi Y, Harrison SC, Walz T. Lipid-protein interactions in double-layered two dimensional crystals of aquaporin-0. *Nature* 2005;438:633–638. [PubMed: 16319884] Advantage is taken of the structure factor amplitudes made available by high quality electron diffraction patterns to determine the structure of aquaporin-0 to a resolution of 1.9 Å. The high resolution enabled precise information to be obtained about each of the nine lipids surrounding the aquaporin-0 monomer
9. Holm PJ, Bhakat P, Jegerschoeld C, Mitsuoka K, Fujiyoshi Y, Morgenstern R, Hebert H. Structural basis for detoxification and oxidative stress protection in membranes. *J Mol Biol* 2006;360:934–945. [PubMed: 16806268]
10. Breyton C, Haase W, Rapoport TA, Kuhlbrandt W, Collinson I. Three-dimensional structure of the bacterial protein-translocation complex SecYEG. *Nature* 2002;418:662–665. [PubMed: 12167867]
11. Ubarretxena-Belandia I, Baldwin JM, Schuldiner S, Tate CG. Three-dimensional structure of the bacterial multidrug transporter EmrE shows it is an asymmetric homodimer. *EMBO J* 2003;22:6175–6181. [PubMed: 14633977]
12. Kukulski W, Schenk AD, Johanson U, Braun T, de Groot BL, Fotiadis D, Kjellbom P, Engel A. The 5 Å structure of heterologously expressed plant aquaporin SoPIP2;1. *J Mol Biol* 2005;350:611–616. [PubMed: 15964017]
13. Oshima A, Tani K, Hiroaki Y, Fujiyoshi Y, Sosinsky GE. Three-dimensional structure of human connexin26 gap junction channel reveals a plug in the vestibule. *Proc Natl Acad Sci U S A* 2007;104:10034–10039. [PubMed: 17551008] This study used electron images of recombinant Cx26 connexin reconstituted in lipids to reveal new architectural details of the gap junction channel: a central plug, in addition to transmembrane helices shaping the channel wall
14. Dubochet J, Adrian M, Chang JJ, Homo JC, Lepault J, McDowell AW, Schultz P. Cryo-electron microscopy of vitrified specimens. *Q Rev Biophys* 1988;21:129–228. [PubMed: 3043536]
15. Unwin N. Acetylcholine receptor channel imaged in the open state. *Nature* 1995;373:37–43. [PubMed: 7800037]
16. Ludke SJ, Serysheva II, Hamilton SL, Chiu W. The pore structure of the closed RyR1 channel. *Structure* 2005;13:1203–1211. [PubMed: 16084392]
17. Signorell GA, Kaufmann TC, Kukulski W, Engel A, Remogy HW. Controlled 2D crystallization of membrane proteins using methyl-beta-cyclodextrin. *J Struct Biol* 2007;157:321–328. [PubMed: 16979348]
18. Vink M, Derr KD, Love J, Stokes DL, Ubarretxena-Belandia I. A high throughput strategy to screen 2D crystallization trials of membrane proteins. *J Struct Biol* 2007;160:295–304. [PubMed: 17951070]
19. Dang TX, Milligan RA, Tweten RK, Wilson-Kubalek EM. Helical crystallisation on nickel-lipid nanotubes: perfringolysin O as a model protein. *J Struct Biol* 2005;152:129–139. [PubMed: 16242343]
20. Cheng A, Leung A, Fellmann D, Quispe J, Suloway C, Pulokas J, Abeyrathne PD, Lam JS, Carragher B, Potter CS. Towards automated screening of two-dimensional crystals. *J Struct Biol* 2007;160:324–331. [PubMed: 17977016]
21. Gibson B, Zeng X, Zhang ZY, Stahlberg H. 2dx-user-friendly image processing for 2D crystals. *J Struct Biol* 2007;157:64–72. [PubMed: 17055742]
22. Philippsen A, Schenk AD, Signorell GA, Mariani V, Berneche S, Engel A. Collaborative EM image processing with the IPLT image processing library and toolbox. *J Struct Biol* 2007;157:28–37. [PubMed: 16919967]

23. Philippsen A, Engel HA, Engel A. The contrast imaging function for tilted specimens. *Ultramicroscopy* 2007;107:202–212. [PubMed: 16989948]
24. Crowther RA, Henderson R, Smith JM. MRC image processing programs. *J Struct Biol* 1996;116:9–16. [PubMed: 8742717]
25. Beroukhi R, Unwin N. Distortion correction of tubular crystals: improvements in the acetylcholine receptor structure. *Ultramicroscopy* 1997;70:57–81. [PubMed: 9440347]
26. Yonekura K, Toyoshima C. Structure determination of tubular crystals of membrane proteins. IV. Distortion correction and its combined application with real-space averaging and solvent flattening. *Ultramicroscopy* 2007;107:1141–1158. [PubMed: 17368944]
27. DeRosier DJ, Moore PB. Reconstruction of three-dimensional images from electron micrographs of structures with helical symmetry. *J Mol Biol* 1970;52:355–369. [PubMed: 5485914]
28. DeRosier DJ. Correction of high resolution data for curvature of the Ewald sphere. *Ultramicroscopy* 2000;81:83–98. [PubMed: 10998793]
29. Egelman EH. The iterative helical real space reconstruction method: surmounting the problems posed by polymers. *J Struct Biol* 2007;157:83–94. [PubMed: 16919474] This paper reports recent developments in the iterative helical real-space reconstruction (IHRSR) algorithm, and its application to a range of helical polymers, where it has been difficult to extract useful information using the traditional Fourier-Bessel approach
30. Pomfret AJ, Rice WJ, Stokes DL. Application of the iterative helical real-space reconstruction method to large membranous tubular crystals of P-type ATPases. *J Struct Biol* 2007;157:106–116. [PubMed: 16879984] A comparison is made of the IHRSR and Fourier-Bessel approaches applied to tubular crystals of membrane proteins. The Fourier-Bessel approach performed better for the well-ordered skeletal Ca^{2+} -ATPase and Na^+/K^+ -ATPase specimens, but not so well for the less well-ordered scallop Ca^{2+} -ATPase
31. Sachse C, Chen JZ, Coureux P-D, Stroupe ME, Fandrich M, Grigorieff N. High-resolution electron microscopy of helical specimens: a fresh look at tobacco mosaic virus. *J Mol Biol* 2007;371:812–835. [PubMed: 17585939] The IHRSR method is applied to the study of tobacco mosaic virus. Some refinements in the method and the inclusion of constraints in the segment alignment, based on the known helical symmetry, led to a density map of about 4.5 Å resolution from only 135 virus particles. An atomic model, derived from this map, revealed details not apparent in the earlier structure obtained by X-ray diffraction
32. Tate CG. Comparison of three structures of the multidrug transporter EmRE. *Curr Opin Struct Biol* 2006;16:457–464. [PubMed: 16828280]
33. Hite RK, Raunser S, Walz T. Revival of electron crystallography. *Curr Opin Struct Biol* 2007;17:389–395. [PubMed: 17723294]
34. Subramaniam S, Gerstein M, Oesterhelt D, Henderson R. Electron diffraction analysis of structural changes in the photocycle of bacteriorhodopsin. *EMBO J* 1993;12:1–8. [PubMed: 8428572]
35. Berriman J, Unwin N. Analysis of transient structures by cryo-microscopy combined with rapid mixing of spray droplets. *Ultramicroscopy* 1994;56:241–252. [PubMed: 7831735]
36. Fujiyoshi Y, Mizusaki T, Yamagishi H, Aoki Y, Kihara H, Harada Y. Development of a superfluid helium stage for high resolution electron microscopy. *Ultramicroscopy* 1991;38:241–251.
37. Nicastro D, McIntosh JR, Baumeister W. 3D structure of eukaryotic flagella in a quiescent state revealed by cryo-electron tomography. *Proc Natl Acad Sci U S A* 2005;102:15889–15894. [PubMed: 16246999]
38. Murphy GE, Leadbetter JR, Jensen GJ. In situ structure of the complete *Treponema primitia* flagellar motor. *Nature* 2006;442:1062–1064. [PubMed: 16885937]
39. Liu J, Taylor DW, Krementsova E, Trybus KM, Taylor KA. Three-dimensional structure of the myosin V inhibited state by cryoelectron tomography. *Nature* 2006;442:208–211. [PubMed: 16625208]
40. Al-Amoudi A, Diez DC, Betts MJ, Frangakis AS. The molecular architecture of cadherins in epidermal desmosomes. *Nature* 2007;450:832–837. [PubMed: 18064004] Cryo-electron tomography of sections through frozen tissue, combined with real-space averaging, is used to elucidate the molecular architecture of cadherins in desmosomes

41. Walz T, Hirai T, Murata K, Smith BL, Heymann JB, Mitsuoka K, Fujiyoshi Y, Agre P, Engel A. Three-dimensional structure of aquaporin 1. *Nature* 1997;387:624–627. [PubMed: 9177353]
42. Cheng A, van Hoek AN, Yeager M, Verkman AS, Mitra AK. Three-dimensional organisation of a human water channel. *Nature* 1997;387:627–630. [PubMed: 9177354]
43. Kim S, Jeon T-J, Oberai A, Yand D, Schmidt JL, Bowie JU. Transmembrane glycine zippers: physiological and pathological roles in membrane proteins. *Proc Natl Acad Sci U S A* 2005;102:14278–14283. [PubMed: 16179394]
44. Senes A, Ubarretxena-Belandia I, Engelman DM. The C α -H-O hydrogen bond: a determinant of stability and specificity in transmembrane helix interactions. *Proc Natl Acad Sci U S A* 2001;98:9056–9061. [PubMed: 11481472]
45. Subramaniam S, Henderson R. Molecular mechanism of vectorial proton translocation by bacteriorhodopsin. *Nature* 2000;406:653–657. [PubMed: 10949309]
46. Singer, SJ. The molecular organization of biological membranes. In: Rothfield, LI., editor. *Structure and Function of Biological Membranes*. Academic; New York: 1971. p. 145-222.
47. Jasti J, Furukawa H, Gonzales EB, Gouaux E. Structure of acid-sensing ion channel 1 at 1.9Å resolution and low Ph. *Nature* 2007;449:316–323. [PubMed: 17882215]
48. Hilf RJ, Dutzler R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* 2008;452:375–379. [PubMed: 18322461]
49. Hollenstein K, Dawson RJ, Locher KP. Structure and mechanism of ABC transporter proteins. *Curr Opin Struct Biol* 2007;17:412–418. [PubMed: 17723295]
50. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science* 1998;280:69–77. [PubMed: 9525859]
51. Beckstein O, Sansom MS. A hydrophobic gate in an ion channel: the closed state of the nicotinic acetylcholine receptor. *Phys Biol* 2006;3:147–159. [PubMed: 16829701]

Figure 1. Protein-lipid arrays used in determining high resolution structures by electron crystallography: two-dimensional crystals (left) and tubular crystals (right). Two-dimensional crystals may contain thousands of unit cells, allowing extensive averaging to improve the signal-to-noise ratio, but need to be tilted to provide the different views required for a three-dimensional analysis. Tubular crystals contain less unit cells, but the molecules are arranged on the surface lattice with helical symmetry, giving rise to many different views and avoiding the need to tilt.

Figure 2.

Portion of a density map of tobacco mosaic virus, and fitted polypeptide chains, determined from images of ice-embedded specimens by the iterative helical real-space reconstruction method (reprinted with permission from [31••]). The density map, at a resolution of about 4.5 Å, was calculated from images of only 135 virus particles.

Figure 3. Domain structures of a ligand-gated ion channel (ACh receptor, pdb accession code: 2BG9; left) and an ABC transporter (ModB₂C₂, pdb accession code: 2ONK, adapted from [49]; right). In these proteins, the interface between the ligand binding (upper) and transmembrane domains is involved in coupling mechanically distinct conformational changes. The ligand binding domain acts as a controlling element to switch the configuration of helices in the membrane. The interfacial regions crucial to the coupling are in black. For clarity, only two of five ACh receptor subunits are shown.

Figure 4.

Comparison of the KcsA potassium channel (pdb accession code: 1BL8, reprinted with permission from [50]; left) with the transmembrane portion of the ACh receptor (pdb accession code: 1OED, adapted from [6]; right), illustrating the importance of precise stereo-chemistry in controlling ion conduction across the membrane. Potassium ions flow readily through the narrow selectivity filter of the KcsA channel, because precisely located carbonyl groups lining its surface coordinate with the ion and substitute ideally for the normally tightly bound water molecules. Despite the much wider diameter, the hydrophobic gate region of the ACh receptor forms an effective permeation barrier to potassium (and other) ions, because the ion has no opportunity to shed its hydration shell and so is, in effect, too large to go through. Space filling representations, with the front subunit removed.