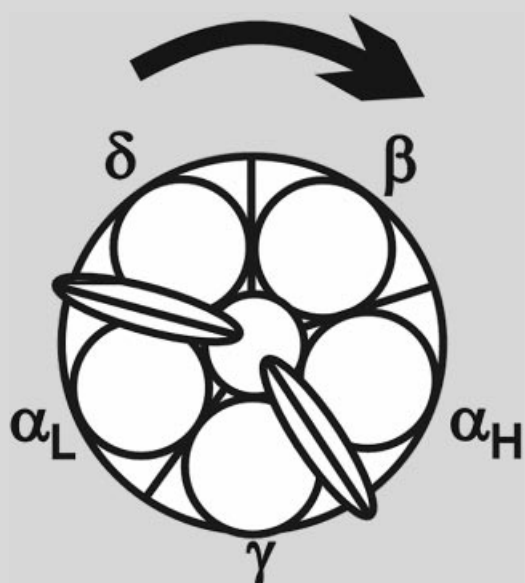
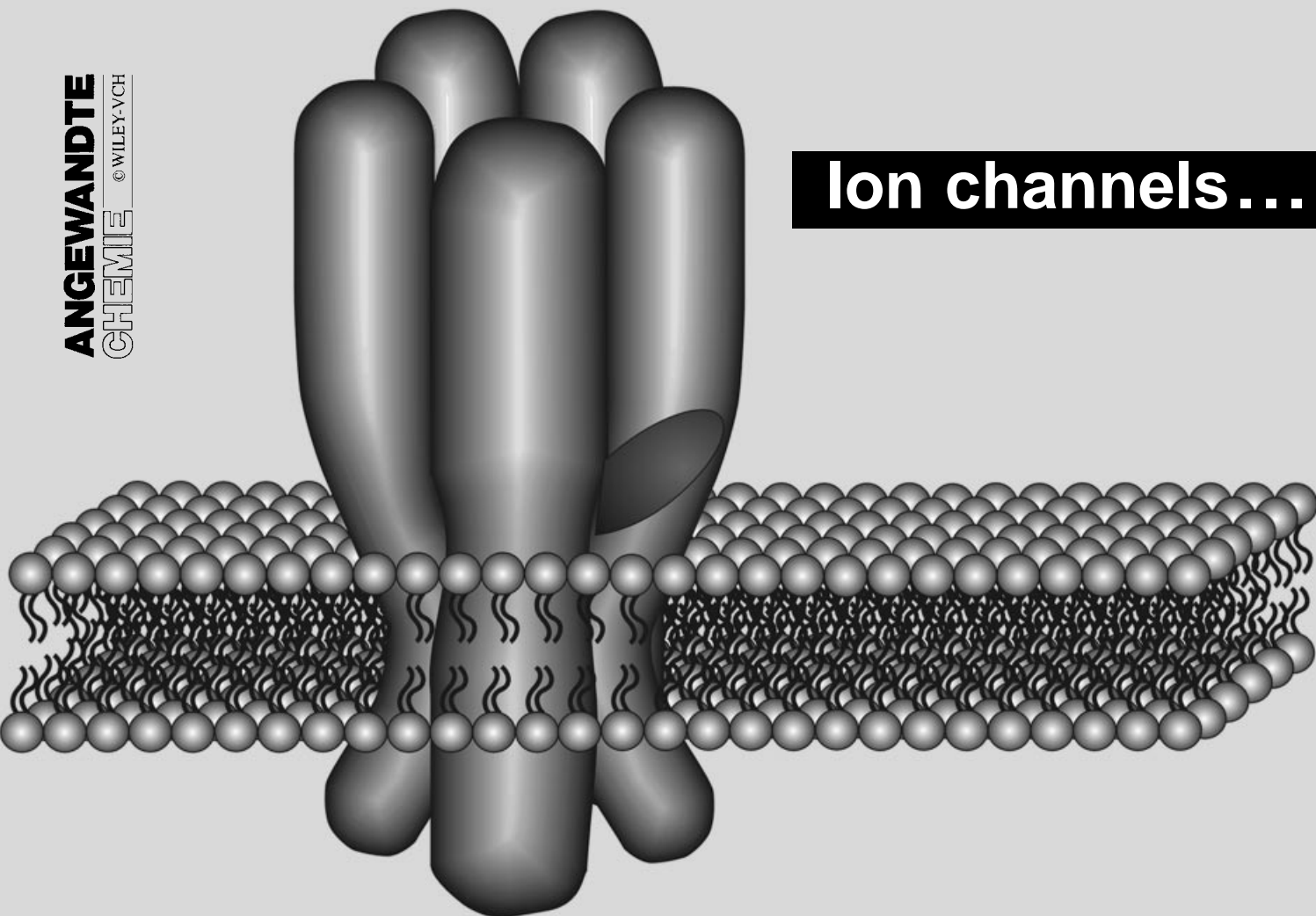
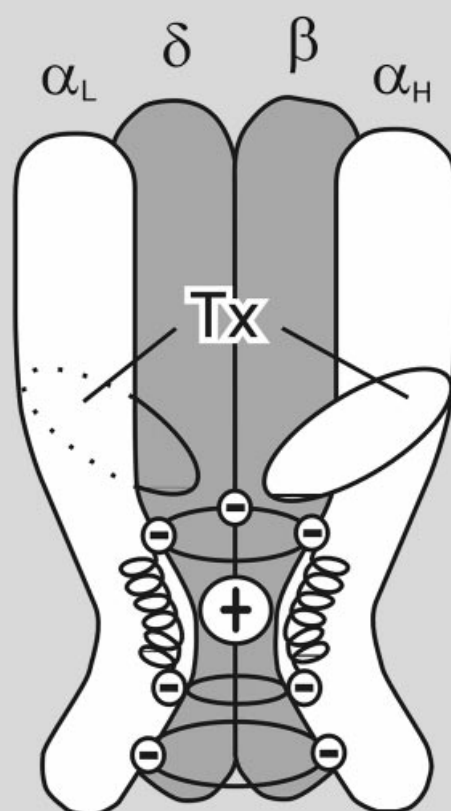


Ion channels...



...are key information-processing proteins



Ligand-Gated Ion Channels

Ferdinand Hucho* and Christoph Weise

Ion channels are at the heart of many biological processes such as nerve activity and muscle contraction. How are their impressive ion selectivity and highly specialized gating brought about? In recent years, X-ray crystallography and high-resolution electron microscopy, as well as photo-affinity

labeling and site-specific mutagenesis techniques in combination with patch-clamp electrophysiology have provided a detailed picture of some channel proteins. Herein we summarize the main structural and functional properties of channel proteins based on the advances made mainly within the last

decade. We integrate these novel insights into a comprehensive description of the class of ligand-gated ion channels.

Keywords: ion channels • neurotransmitters • proteins • receptors • structure elucidation

1. What are Ion Channels and What Purpose Do They Serve?

Probably all living cells exhibit a membrane potential at their plasma membrane. This diffusion potential is caused by the unequal distribution of ions between the inside and outside of the cell, and by the membrane's semipermeability. The value of the potential is given by the Goldman–Hodgkin–Katz Equation [Eq. (1)], an extended version of the Nernst equation. In Equation 1, R is the gas constant, T is the temperature in Kelvin, F is the Faraday constant, and P_x , $[X]_o$, and $[X]_i$ are the relative permeabilities and the external and the internal concentrations of the respective ions.

$$E = \frac{RT}{F} \ln \frac{P_{\text{Na}}[\text{Na}^+]_o + P_{\text{K}}[\text{K}^+]_o + P_{\text{Cl}}[\text{Cl}^-]_i}{P_{\text{Na}}[\text{Na}^+]_i + P_{\text{K}}[\text{K}^+]_i + P_{\text{Cl}}[\text{Cl}^-]_o} \quad (1)$$

Nerve cells (neurons) take advantage of their membrane potential for intracellular and intercellular communication: local changes in the potential, the so-called electrotonic potentials, are the basis for signal processing and triggering of intracellular responses. When they exceed certain threshold values, they also trigger action potentials. These are transient, large depolarizations and repolarizations of excitable membranes which travel over long distances along the neuronal membrane until they reach their target. The nerve ending together with the membrane of the neighboring cell form a synapse. At the nerve terminal, the depolarization releases specific chemicals, the neurotransmitters, which in turn

depolarize the membrane of the postsynaptic cell. There again, local or action potentials may be the result.

Changes in membrane potentials that underlie both local and action potentials are caused by ion currents across the membrane. Since the lipid bilayer that forms the plasma membrane poses an immense energy barrier for charged particles, specialized transmembrane proteins carry the ion currents. These proteins are the ion channels, the topic of this article.

Ion channels have several unusual features, which make them the subject of much biochemical, biophysical, and physiological research. For one, they are highly selective. They discriminate not only between anions and cations, but even between different monovalent and divalent ions, for example, Na^+ , K^+ , and Ca^{2+} . Another important feature is their response to specific signals: at rest they are tightly closed and impermeable, but they are opened (“gated”) either by changes in the membrane potential or by certain ligands such as the neurotransmitters. According to this description, all ion channels are composed of two functional moieties (Figure 1),

- a **selectivity filter (S)**, which determines which types of ions may pass the membrane; and
- a **gate (G)**, which specifies under which conditions the channel is opened.

Channels are subdivided into two major classes according to their gating trigger (L): the voltage-gated channels (VGCs) and the ligand-gated channels (LGCs).

2. Classification of Ligand-Gated Ion Channels

Both classes of channels, the VGCs and the LGCs, are diverse and comprise numerous members. Here it is neither

[*] Prof. Dr. F. Hucho, Dr. C. Weise
Institut für Chemie – Biochemie
Freie Universität Berlin
Thielallee 63, 14195 Berlin (Germany)
Fax: (+49) 30-8385-3753
E-mail: hucho@chemie.fu-berlin.de

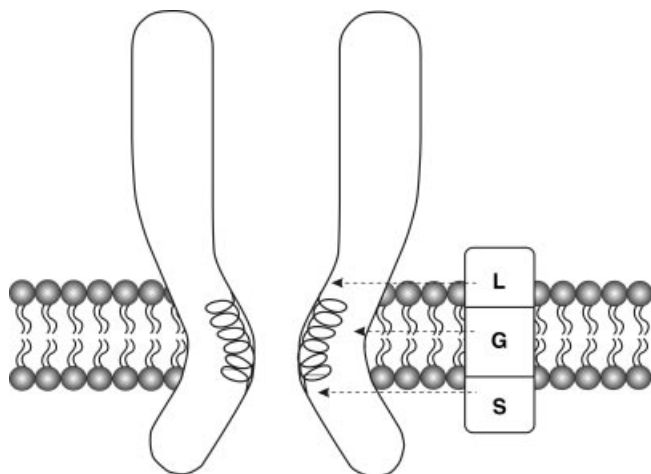


Figure 1. Schematic representation of ligand-gated ion channels. Longitudinal cross section of the membrane-spanning protein depicting the wide extracellular vestibule (top) and the intramembrane structure; the channel is lined with transmembrane helices and contains the channel gate (G) and selectivity filter (S). The ligand-binding site (L), which triggers the gating by an allosteric mechanism, is located on top of G and S.

intended nor possible to give a comprehensive survey covering them all. We rather want to focus on a few general principles of ion-channel structure and function, based on recent crystallographic and molecular biological data. After briefly touching on other channels, we turn to the neurotransmitter receptors as the pharmacologically most important and best-known class of LGCs. By definition, this limits our treatise to LGCs of the animal kingdom, although it should be mentioned that both VGCs and LGCs also occur in microorganisms, in unicellular eukaryotes, and even in plants.

For example, several genes that code for ionotropic glutamate receptors have been discovered in *Arabidopsis*;^[1] they might have been present before plants and animals diverged.^[2]

The term “ionotropic” refers to the ion-channel nature of the receptor and indicates that there is a different type, the metabotropic receptors. These are “seven-transmembrane(7TM)-sequence receptors”. Most neurotransmitters, with the exception of glycine, act through both ionotropic and metabotropic receptors. The 7TM receptors, which signal by coupling to G-proteins and second-messenger-producing enzymes, are functionally and mechanistically completely different and are not discussed further in this review.

A simple classification emerged as a result of the cloning and sequencing of many LGCs. It turned out that several LGCs, although different in function, have similar amino acid sequences. According to this criterion, LGCs can be classified into three superfamilies (Figure 2).

1. The superfamily of receptors that resemble the nicotinic acetylcholine receptors: glycine receptors (GlyR), GABA_A receptors (GABA_AR), nicotinic acetylcholine receptors (nAChR), and some serotonin receptors (5-HT₃R).
2. The superfamily of ionotropic glutamate receptors (GluR).
3. The ATP-gated purino receptors (P2X).

A “family” is defined as receptors that are coded by distinct though similar genes and that react through basically the same mechanism to the same neurotransmitter. A “superfamily” is a set of receptor families that are diverse in function and mechanism, most often reacting to different neurotransmitters, but seem to evolve from a common ancestor. Besides similarities in their sequences, they have conserved several structural features.

Ferdinand Hucho studied Chemistry in Freiburg, where he obtained his Dr. rer. nat. in 1968 with work on enzyme kinetics under the supervision of Kurt Wallenfels. His postdoctoral work was on the regulation of multienzyme complexes at the University of Texas at Austin with Lester Reed. In 1973 he received his habilitation in Biochemistry at the Universität Konstanz. In the same year he changed his field, moving from metabolic regulation to neurochemistry. At the Pasteur Institute in Paris (with Jean-Pierre Changeux) he started working on receptor proteins and signal transduction. In 1979 he became Professor of Biochemistry in Konstanz; in the same year he moved to the Freie Universität Berlin. In 1988/89 he was offered the Chair of Biochemistry in Giessen, Cologne, and Bochum; however, with the collapse of the Berlin Wall he decided to stay in Berlin. He became a member of the Berlin–Brandenburg Academy of Science in 1996.



F. Hucho



C. Weise

Christoph Weise was born in 1956 in Hof, South Germany. He received his Diploma in Biochemistry (1985) and Ph.D. (1991) from the Freie Universität Berlin, where he worked on acetylcholinesterase in the neurochemistry unit under the direction of Ferdinand Hucho. Since then, he has been a research assistant at the Institut für Chemie-Biochemie of the Freie Universität Berlin, where he is in charge of a protein analysis facility and teaches university courses on advanced protein chemistry to biochemistry majors. His research involves the acetylcholine receptor and he has continued his work on acetylcholinesterase during research stays at the Institut Pasteur, Paris and at INRA, Montpellier.

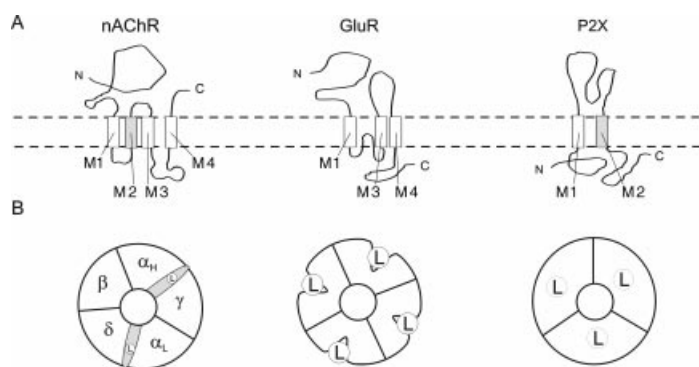


Figure 2. Transmembrane folding (A) and quaternary structure (B) of three representative superfamilies of ligand-gated ion channels. M1 to M4 represent the transmembrane sequences, and N and C represent the amino and carboxy termini of the protein, respectively. L marks the binding pockets for the respective receptor ligands; note that for the nAChR it is depicted as spanning the receptor complex from the outside to the lumen of the channel. The P2X stoichiometry shown is preliminary at this point, as it was determined with a heterologously expressed channel protein.^[5]

The members of the receptor superfamily 1 (Figure 3) all have similar peptide loops formed by disulfide bridges, glycosylation patterns, distribution of proline, and (in one specific domain which forms the channel wall) Ser/Thr residues. Most conspicuously, all the receptors of this superfamily possess four hydrophobic amino-acid sequences, which are long enough to span the plasma membrane. Accordingly, they sometimes are termed “four-transmembrane(4TM)-sequence receptors”.

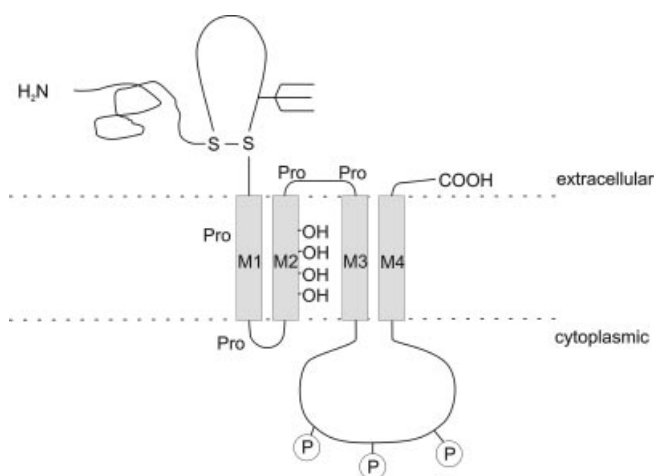


Figure 3. Schematic representation of the transmembrane folding of subunits of the receptors that resemble the nicotinic acetylcholine receptors, showing the position of a characteristic disulfide bridge (-S-S-), proline residues (Pro), and side chains with hydroxy groups (-OH) lining the channel-forming transmembrane helices M2. The trident marks the position of oligosaccharide structures. M3 and M4 are linked by a large cytoplasmic loop which is the target of various protein kinases that introduce phosphate residues (P).

The members of the glutamate receptor superfamily presumably have three transmembrane sequences. A fourth hydrophobic sequence enters and leaves the plasma membrane from the cytoplasmic side, thereby forming a loop (re-entrant loop), which is presumed to line the channel (see

Section 4.1.2). The ATP-binding P2X receptors are thought to contain only two transmembrane (2TM) sequences.

Receptors that belong to the same superfamily are presumed to have similar tertiary and quaternary structures. As shown in Figure 2B, the receptors that resemble the acetylcholine receptors are pentamers,^[3] whereas glutamate receptors are probably tetramers.^[4] It was shown for the P2X receptors that heterologously expressed receptors tend to form stable homotrimers.^[5]

To complete this introductory overview, it should be mentioned that there are other LGCs, such as the cyclic-nucleotide-gated (cNMP) channels (e.g. in the retina of the eye and in the olfactory system) and the calcium-release channels. The latter channels are gated by inositol trisphosphate (IP₃) or by ryanodine, and they regulate the calcium permeabilities of the endoplasmic reticulum and of the t-tubule membrane of skeletal muscle, respectively. The different types of ion channels are related: recently it was shown that TRP channels, a special family of calcium channels that mediate calcium influx into cells, also called store-operated Ca²⁺ channels, may even be gated by another LGC, the IP₃ receptor.^[6, 7] Another family of LGCs are the vanilloid receptors (VR), which are involved in nociception. These VRs are gated by protons (pH < 6.0) and heat (> 42 °C),^[8] and also exhibit little structural and mechanistic similarity to the neurotransmitter-gated receptors discussed herein.

3. Some Features of LGCs

Before we discuss two particularly well-known LGCs in more detail, we would like to summarize some principles of the functions of neurotransmitter receptors:

3.1. Sources of Diversity

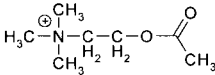
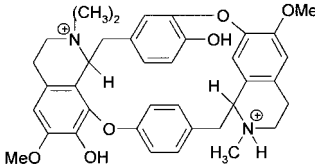
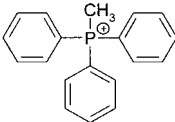
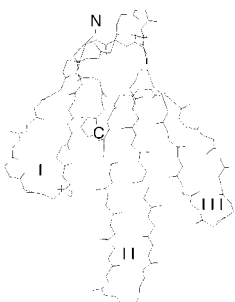
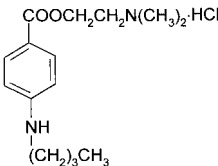
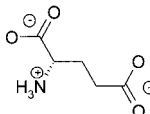
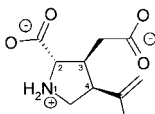
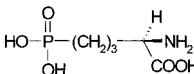
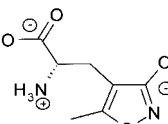
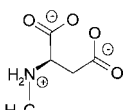
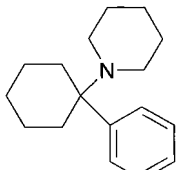
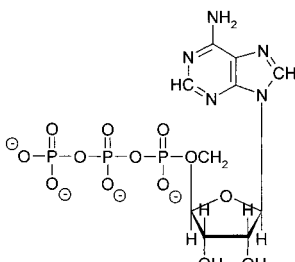
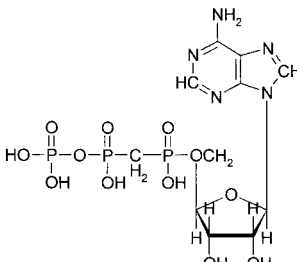
The functional diversity observed in the central nervous system is not brought about by the use of numerous transmitters; their number is limited to maybe a dozen. Rather, this diversity comes from the multiplicity of receptors. For many of the LGCs, a quaternary structure formed from a number of distinct but homologous subunits has been reported.^[9] The molecular cloning of the neuronal nicotinic receptors has revealed that these receptors are very complex because of the existence of multiple subunits. For each receptor there is a large family of related genes that code for subunits, which assemble in a combinatorial manner to form functionally different receptors. Thus 11 different subunits were found for neuronal nAChRs, 5 for peripheral nAChRs, 16 for GluRs, and at least 17 for GABA_ARs.^[10] The latter family can be subdivided into six classes of subunits (α, β, γ, δ, ε, ρ), which have about 20 to 30% sequence identity between and 70% within the classes. Assemblies of most of these subunits have been reported in the vertebrate brain. For the GluRs, even more variants can arise from alternative splicing, and in the case of particular receptor subtypes (see Section 4.1.2), by RNA editing.^[11, 12] The functional importance of this phenomenon will be explained in the chapter on glutamate receptors.

3.2. Regulation

Diverse Receptor Ligands

The specific stimulus for the channel opening of the LGCs is the binding of a specific neurotransmitter, but they can also be influenced by a variety of substances besides their physiological ligand. Some of these compounds are given in Table 1. According to their function, two different groups are distinguished: agonists initiate the same response as the physiological ligand (e.g. nicotine is an agonist of the nAChR) and antagonists oppose the effect of an agonist, normally by occupying the same binding sites with similar affinities as agonists. The reason why agonists and antagonists have such different effects is generally not known. Many naturally

Table 1. A selection of ligands specific for the superfamilies of ligand-gated ion channels.

Super-family	Agonist	Competitive antagonists	Noncompetitive antagonists (NCIs)
Ⓐ <i>n</i> AChR	 <p>Acetylcholine</p>	 <p>(+)-Tubocurarine</p>	 <p>TPMP⁺</p>
		 <p>α-Neurotoxin</p>	 <p>Tetracaine</p>
Ⓑ Glu	 <p>Glutamate</p>	 <p>Kainate</p>	 <p>D-AP5</p>
	 <p>AMPA</p>	 <p>NMDA</p>	 <p>Phencyclidine</p>
Ⓒ P2X	 <p>ATP</p>	 <p>α, β-Methylene-ATP</p>	

occurring plant and animal toxins are antagonists of LGCs. For example, strychnine binds to the GlyR, and (+)-tubocurarine and snake venom α -toxins bind to the nAChR. In many cases, such toxins are valuable tools for biochemical research (reviewed in ref. [13]).

A third group of receptor ligands are the noncompetitive inhibitors (NCIs), a structurally heterogeneous group of compounds that bind to distinct sites on the receptor surface. They either act allosterically or in many cases bind to high-affinity sites within the ion channel, thereby sterically blocking the ion flux (channel blockers). These substances can be used to probe the structure of ion channels. NCIs include drugs such as local anesthetics and neuroleptics as well as naturally occurring alkaloids such as frog toxins.

The picture is further complicated by the existence of a pharmacologically very interesting group of substances called inverse agonists. These compounds act at the same receptor sites as agonists, yet produce an opposite effect. Such compounds have been described for the benzodiazepine-binding site on the GABA_AR. Benzodiazepines themselves are widely used tranquilizers that exert an anxiolytic effect. The esters of β -carbolines act as inverse agonists of benzodiazepines, not only blocking their effect, but provoking opposite behavioral actions, that is, they produce signs of anxiety and cause sleeplessness and seizures.

Allosteric Regulation

In many systems, a separate protein functions as the transducer between the receptor site and the effector site—in the case of the 7TM receptors, the transducers are G-proteins. In the case of the LGCs, however, no such coupling protein exists, and the receptor domain (ligand-binding site) and the effector (ion channel) communicate through conformational changes within the receptor only. The energy necessary to trigger this process comes from the ligand-binding energy.

The LGCs are allosterically regulated proteins, that is, they regulate their activity by shifting the equilibrium between different functional states that correspond to distinct conformational states. For example, the nAChRs show classic allosteric behavior: both ligand binding and channel opening are cooperative processes, evidence for which is provided by sigmoidal dose/response and binding curves (summarized in ref. [14]).

Phosphorylation

Another level of regulation is added by post-translational modifications. The LGCs contain multiple phosphorylation sites that have been mapped to their cytosolic loops; these are phosphorylated by different enzymatic activities.^[15] The best characterized system is the nAChR of the neuromuscular junction: agrin stimulates the phosphorylation of tyrosine residues (reviewed in ref. [16]), whereas phosphorylation of serine residues is stimulated by the neuropeptide CGRP (through protein kinase A) and by acetylcholine (through Ca²⁺-dependent kinases). Although a regulation of the channel activity was observed under artificial conditions—for example, the rate of desensitization of the muscle nAChR

is altered by phosphorylation—an *in vivo* regulation was not convincingly shown. Clearly these modifications have the potential to modulate synaptic efficiency, but their functional significance is far from clear.

Protein–Protein Interaction

LGCs interact with proteins inside the cell. The GlyR is attached to cytoskeletal elements through the peripheral membrane protein gephyrin.^[17] The proteins rapsyn and GABARAP appear to fulfil this task for the nACh and GABA_A receptors, respectively.^[18] Different subtypes of glutamate receptors are linked to specific scaffold proteins of the postsynaptic density, for example, PSD-95 or GRIP.^[19, 20] In this way, extensive protein complexes are formed near the neuronal surface (reviewed in ref. [21]). These interactions seem to be the basis for localized clustering and subcellular targeting of LGCs, thus regulating their activity and post-translational modifications, but raising the possibility of direct signalling into the cell. A signalling complex that involves the NMDA receptor was recently isolated from rats and consists of at least 77 proteins.^[22]

Regulation of Biosynthesis

The biosynthesis of the LGCs is tightly regulated. The process is best understood for the nAChR during synapse formation at the neuromuscular junction (reviewed in ref. [23]). The high local receptor density at subsynaptic locations is brought about by both presynaptic factors (ingrown motoneurons) and postsynaptic factors (electrical activity). First, receptors that are initially diffusely distributed over the entire cell surface are directed to the subsynaptic zone. Subsequently, the transcription of the nAChR genes is enhanced only in those nuclei that are close to the developing synapse by means of a mechanism that involves presynaptically released proteins such as agrin and neuregulin. In contrast, transcription is downregulated in extrasynaptic nuclei in response to depolarization (receptor activation).

3.3. LGCs and Disease

Because of their capacity to modify synaptic function, one can expect that LGCs play an important role in various mental disorders, for example, schizophrenia, epilepsy, or depression, and that drugs that act on the LGCs can be used to treat these. Mutations in LGC genes that underlie a number of inheritable human diseases have been found (listed in ref. [24]), mainly by positional cloning: mutations in the nAChRs of the neuromuscular endplate that compromise normal transmission are the basis for congenital myasthenic syndrome,^[25] whereas mutations in the gene of the neuronal α 4 nAChR subunit cause different conditions such as benign familial neonatal convulsions,^[26] or a partial form of epilepsy called autosomal dominant nocturnal frontal lobe epilepsy.^[27] Hyperekplexia (Startle disease) results from a defect in the

glycine receptor gene GLRA1.^[28] Moreover, anti-nAChR antibodies cause the autoimmune disease *Myasthenia gravis* (severe muscle weakness).^[29]

4. Individual Representatives of LGC Families

We choose two LGCs for a more detailed description: the glutamate receptors (GluR) and the nicotinic acetylcholine receptors (nAChR). Neither has been crystallized yet, but we know enough molecular detail to address the following basic questions:

- What is the molecular basis of the impressive ion selectivity (the selectivity filter)?
- What is the structural correlate of the gating mechanism?

In the case of the LGCs, this question includes the molecular prerequisite of selectivity for the triggering signal, that is, the structure of the neurotransmitter-binding pocket, and the coupling between binding and gating.

4.1. Glutamate Receptors

4.1.1. The “Family”, Function, and Structure

Glutamate is the main excitatory neurotransmitter in the brain. It acts through both metabotropic and cation-selective

ionotropic glutamate receptors. The latter form a large family of ligand-gated ion channels, including the AMPA, kainate, and NMDA receptors (AMPA stands for α -amino-3-hydroxy-5-methyl-4-isoxazole propionate; kainate, a natural product, is an excitotoxic glutamate analogue produced by an algae; NMDA is *N*-methyl-D-aspartate; see Table 1). Although all three subtypes respond to glutamate, they can be distinguished by their response to artificial agonists. Their distribution in the brain, physiological function, and mechanism and kinetics of activation and regulation are very different. We do not intend to duplicate herein the many comprehensive reviews on glutamate receptors,^[30–32] but only to summarize some of the structural information necessary to discuss in detail the two basic questions specified above.

The primary structures of many if not all glutamate receptors, including several splice variants, have been deduced from their cloned and sequenced cDNA. For their nomenclature and evolutionary relation, see Figure 4. As is the case with the receptors that resemble nicotinic acetylcholine receptors, the GluR subunits contain four conspicuous hydrophobic sequences, but in contrast to the 4TM receptors, they are presently presumed to span the membrane only three times (Figure 5). The second hydrophobic sequence was shown to enter and exit the plasma membrane at the cytoplasmic surface, thus forming two antiparallel β -strands that line the channel. Several (probably four) of these polypeptide chains form homo- or hetero-oligomeric quaternary structures.

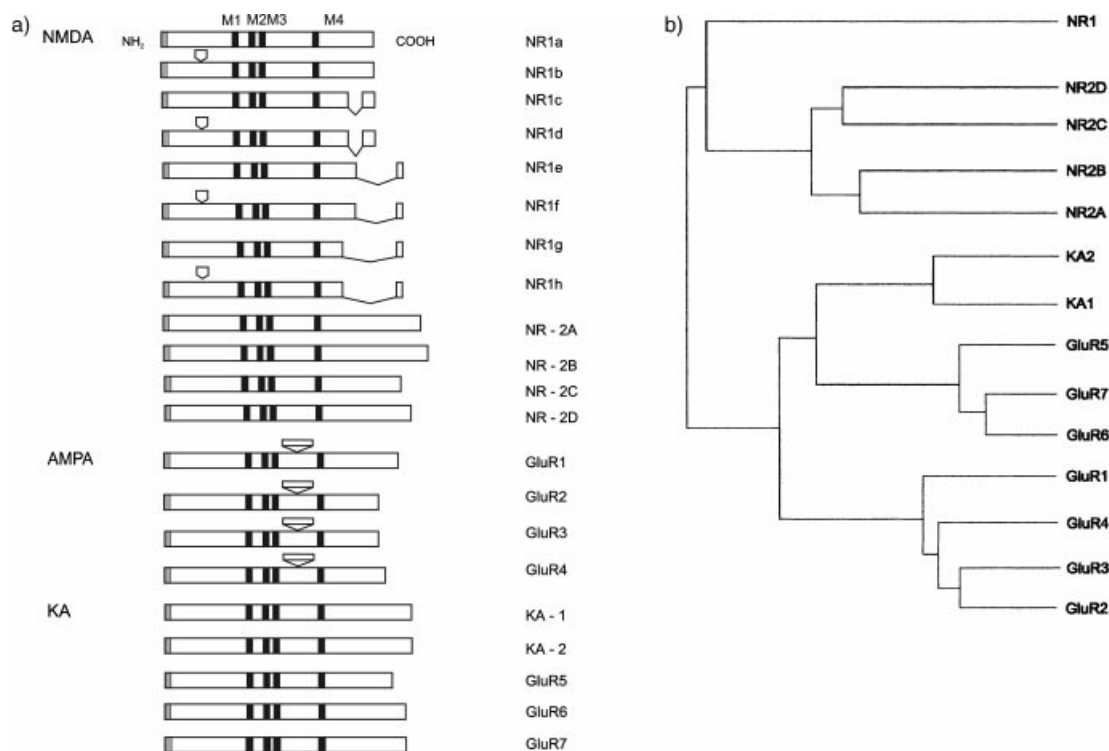


Figure 4. Heterogeneity of glutamate receptors. a) Linear representation of the structure of ionotropic glutamate receptors; GluR stands for the AMPA and kainate subtypes, NR represents the NMDA receptors, and KA represents the kainate-binding proteins. The black boxes represent the hydrophobic sequences M1–M4, and the hatched box at the NH₂ terminus depicts the signal peptide. Variants arise from exonic splicing; the arrows above the diagram indicate splice inserts. The V-shaped lines are for insertions/deletions introduced to obtain an optimal alignment between the sequences. Figure and legend modified from ref. [30]; b) evolutionary tree of glutamate receptors: starting from a common ancestor (left) the actual variety of glutamate receptors (right) with different pharmacological profiles and functional properties has evolved.

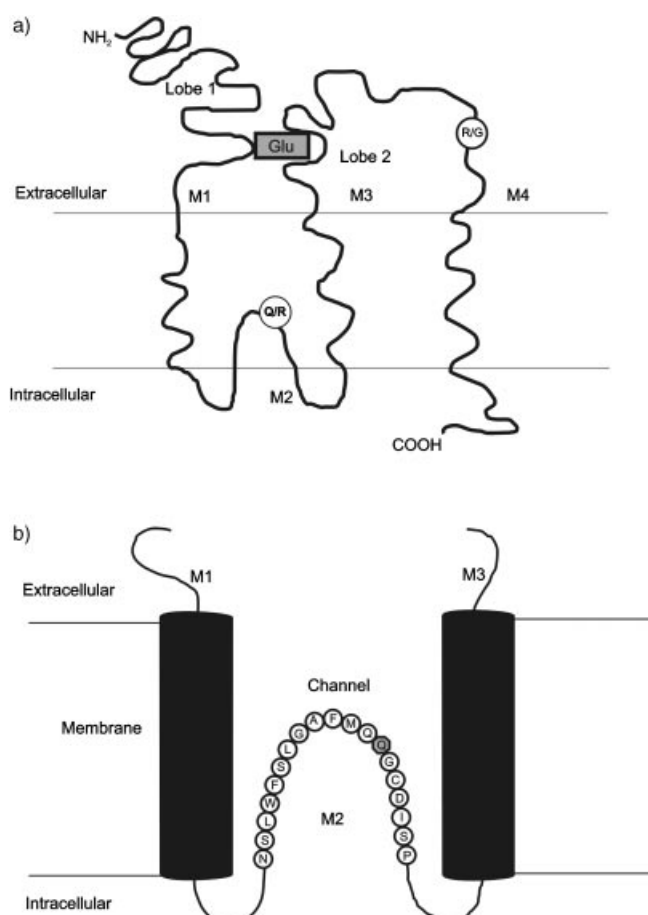


Figure 5. Functional domains of glutamate receptors. a) The agonist-binding site between lobe 1 and lobe 2 of the folded polypeptide chain; M2 represents the re-entrant loop that forms the ion channel; Q/R is the site of modification through RNA editing which affects ion selectivity; b) close-up of M2; the shaded glutamine residue (Q) is replaced by arginine after RNA editing. (Figure modified from ref. [30].)

4.1.2. The Selectivity Filter: Lessons from a Bacterial Channel

Before discussing the channel structure of glutamate receptors, we have to turn to the voltage-gated ion channels, because one of these, the potassium channel KcsA from the bacterium *Streptomyces lividans*, has been crystallized and gives us for the first time an ion channel structure with 3.2-Å resolution (Figure 6).^[33] Some of its features allow us to understand the ion selectivity of voltage- and possibly of ligand-gated ion channels as well, and channel-mediated ion conduction through the membrane lipid phase.

We have good reasons to use this prokaryotic channel as a model for eukaryotic glutamate receptors: an evolutionary missing link was recently detected, the glutamate receptor GluR0 from the cyanobacterium *Synechocystis*.^[34] This protein has a sequence that is similar to eukaryotic glutamate receptors as well as to potassium channels. It is a potassium-selective LGC and contains even the signature sequence TVGYGD characteristic of potassium channels. The KcsA channel is formed by four identical polypeptide chains, each of which contains two-membrane-spanning sequences (2TM) and one re-entrant loop called P. As is the case with

glutamate-receptor channels, the P loop lines the pore and contains the determinants for ion selectivity and conduction.

Potassium channels are slightly permeable to the larger Rb^+ and virtually impermeable to the smaller Na^+ and Li^+ ions. This indicates that channels are not just holes that filter ions of a given radius. On the contrary, they all conduct small ions that have been stripped of their hydration shell, and the selectivity is a result of their ability to remove the water dipoles surrounding the charged particle (Figure 7). The K^+ selectivity filter is lined by carbonyl oxygen atoms. "To compensate for the energetic cost of dehydration, the carbonyl oxygen atoms must take the place of the water oxygen atoms, come in very close contact with the ion, and act like surrogate water. ... a K^+ ion fits in the filter precisely so that the energetic costs and gains are well balanced".^[33] For the smaller Na^+ , the carbonyl oxygen atoms are too far apart to provide enough "energetic gain" to compensate for the cost of dehydration.

This ingenious mechanism poses a major problem: how can a large flux of cations be secured if they are complexed so tightly in the selectivity filter? This problem is overcome by an equally ingenious mechanism: there are two cations simultaneously in the selectivity filter (Figure 7b). Their close proximity and "repulsion overcomes the otherwise strong interaction between ion and protein and allows rapid conduction in the setting of high selectivity".^[33]

We shall recognize the theme of protein oxygen atoms in the selectivity filter of the LGCs discussed in Section 4.2.2. Another feature postulated for the LGCs which was proven by the X-ray crystallographic study of the microbial K^+ channel is a wide water-filled entrance to the channel. In the case of the K^+ channel, a deep largely hydrophobic cavity precedes the selectivity filter (which occupies only 12 Å of the membrane's thickness). This cavity is wide enough to be filled with water and to accept the cation in its hydrated form. Thus the cation can traverse most of the membrane easily; the electrostatic barrier posed by the membrane is lowered, thus ensuring a low-resistance pathway up to the selectivity filter. Experimental proof is simple: TEA (tetraethylammonium) is a selective blocker of potassium channels. It can enter and plug the cavity, because it cannot shed its "ligands" like potassium can do with its hydration shell.

For the channels of GluRs, we have to resort to site-directed mutation experiments in combination with patch-clamp electrophysiology instead of the more direct evidence given by an X-ray crystallographic map. These types of experiments have shown that a re-entrant loop similar to the potassium channel's P loop lines the channel and forms the selectivity filter (Figure 5).^[35] Interestingly, one amino acid residue in this loop determines whether or not the channel is permeable to calcium ions. One specific asparagine (N) residue in the P loop of the NMDA receptors is responsible for their calcium permeability. Nature takes advantage of this key residue: the homologous position in AMPA receptors is occupied by a glutamine (Q) residue; if this residue is converted into an arginine (R) group (specifically in the GluR2), the channel becomes virtually impermeable to calcium ions. This Q/R conversion is brought about by a rare mechanism, namely RNA editing (reviewed in ref. [36]): in the pre-mRNA, the



Figure 6. Stereoview of the X-ray structure of the potassium channel KcsA.^[33] Top: the fold of the KcsA tetramer viewed from the extracellular side. Bottom: the channel seen from a perpendicular perspective. (Figure courtesy of Roderick MacKinnon; reproduced with permission of *Science* and the authors).

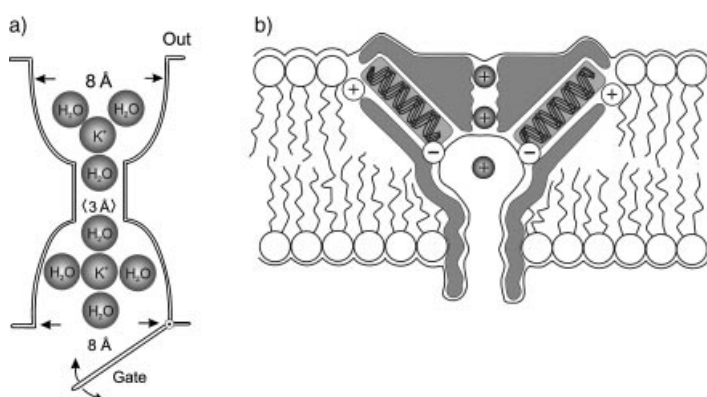


Figure 7. The ion pathway through voltage-gated ion channels with structural features similar to the ligand-gated ion channels. a) Historical representation of potassium channels which indicate the diameter of the intracellular and extracellular vestibule and the selectivity filter, which allows the passage only of partially dehydrated cations; b) the modern view deduced from X-ray crystallographic analysis (see text). (Figure adapted from ref. [33].)

codon CAG for glutamine is deaminated enzymatically to CIG (adenosine to inosine, catalyzed by an adenosine desaminase). One would like to see the consequences of this Q/R conversion on a protein map with atomic resolution, but unfortunately a crystal structure is not available yet.

To understand the gating mechanism, we had to resort to KcsA as a model for LGCs. With the glutamate receptor's ligand-binding site, which confers the specificity of the signal and the triggering mechanism for the channel gating, we are better off, since a 3D structure of this domain of the transmitter receptor itself is available. The glutamate receptor's ligand-binding domain has been crystallized and its structure, when complexed with the toxic agonist kainate, has been solved with high resolution.^[37] Previously, site-directed mutagenesis experiments had shown that the ligand-binding domain of GluRs is formed by two noncontinuous sequences, S1 and S2.^[38, 39] S1 includes about 150 N-terminal amino acids, ending with TM1, and S2 links TM3 with TM4. The fusion of S1 to S2 with a short peptide by means of recombinant DNA techniques yields a water-soluble protein, which was expressed in bacteria in large quantities.^[40, 41] This artificial binding domain has ligand-binding and pharmacological properties very similar to the natural NMDA receptor. An X-ray crystallographic study of this expression product yielded an overall

structure similar to a Venus flytrap (Figures 6 (top) and 9): the two lobes come close together to trap the ligand (kainate). Interestingly, this Venus flytrap model closely resembles the glutamine-binding protein QBP from bacteria, which shows only little sequence similarity to GluRs.^[42]

Amino acid residues of both lobes participate in ligand binding. Kainate binds deep down in a gorge formed by the two lobes. Each lobe points two helices with its amino terminus (the positive end of their dipoles) towards the negatively charged carboxy group of the agonist. Binding pulls the lobes together thereby closing the trap. Together with previous mutagenesis experiments, the residues that determine the binding affinity and specificity can now be identified (Figure 8).^[43–45] For example, the 2-carboxy group of kainate binds to an arginine residue (R485 in GluR2) and to the NH group of a threonine residue (T480) in the polypeptide backbone.

More recent experiments in which a similar GluR construct was cocrystallized with various ligands have shown for the

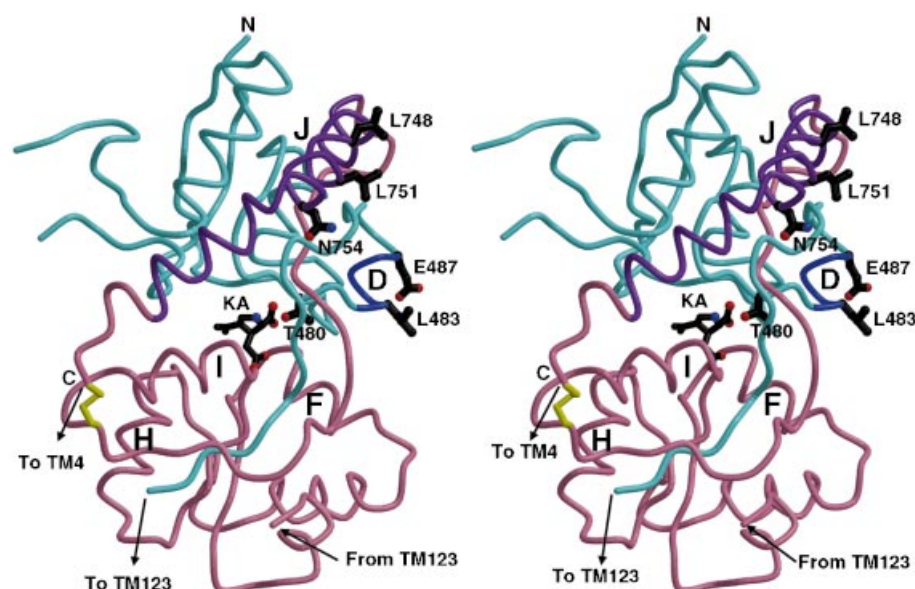


Figure 8. Stereoview of the high-resolution structure of the ligand-binding site of the AMPA-subtype glutamate receptor, cocrystallized with kainate (KA). The upper lobe (represented in turquoise and violet) together with the lower lobe (mauve) form the Venus flytrap structure. (Reproduced courtesy of Eric Gouaux.)

first time the mechanism underlying activation and antagonism: the extent of ligand-induced activation depends on the degree of domain closure, and the degree to which the two domains in the flytrap model are contracted depends on the nature of the ligand.^[46] Agonists cause domain closure, which eventually leads to channel activation. The binding of partial agonists results in correspondingly smaller conformational changes, whereas antagonists act by freezing the ligand-binding core in an open-cleft conformation.

4.1.3. The Gating Mechanism

Once again we resort to the microbial KcsA to learn how channel gating occurs, although its physiological relevance is not clear and one should be aware that it might act very differently from LGCs. The KcsA potassium channel of *Streptomyces lividans* can be stabilized in the open state at low extracellular pH values. Protons seem to be the “ligands” that trigger the gating. The transmembrane helices TM1 and TM2 of the four channel subunits form the pore. The selectivity filter composed of the four P loops is located at the upper (extracellular) end, but where do we look for the gate? Based on a combination of ion-flux experiments, and CD, FTIR, and EPR spectroscopic investigations of wild-type and mutated channels, including inward-rectifier channels from eukaryotes (summarized in a series of reviews, see ref. [47]), the following model has been proposed: during gating, conformational changes along TM2 occur. They are not caused by large changes of secondary structure, nor are they restricted to the intracellular C-terminal ends of the helices (where the gate of voltage-gated K⁺ channels is localized). Both TM1 and TM2 as a whole tilt slightly and rotate counterclockwise. This “rigid-body movement” causes a widening of the gate, as depicted schematically in Figure 9.^[48] The helices behave as

rods that open and close the pore. The “signature sequences” (the selectivity filter) experience no significant movement. As we will discuss in Section 4.2.2, similar minute movements may underlie the gating of LGCs. This mechanism is very different from the situation observed in other voltage-gated potassium channels (discussed in ref. [49]), in which, for example, a channel-occluding plug is removed upon activation (“ball-and-chain” mechanism).

It should be pointed out that much of the dynamics of channel gating discussed in this section is based on indirect biophysical evidence and on static X-ray crystallographic images; therefore it should be considered speculative and needs further experimental support.

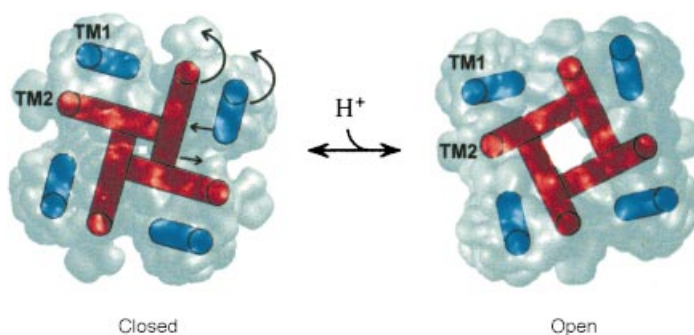


Figure 9. Proposed model of a molecular activation mechanism for the gating of a voltage-dependent potassium channel by a rigid-body movement; view of the KcsA channel (see text) from the extracellular side. Upon activation, the counterclockwise movement of the transmembrane helix TM2 away from the permeation pathway opens the channel. For illustration purposes, the movements depicted are larger than expected and constitute a simplified representation. (Reprinted from ref. [48] with kind permission of *Science* and the authors.)

4.1.4. Crystallographic Proof of the Reliability of Biochemical and More Indirect Methods of Structure Determination

The first X-ray crystallographic analysis of an important ligand-gated channel domain proves above all one important point in this whole field of research: crystallography and mutation research are not only complementary methods; the vast amount of information collected by biochemical and biophysical research, including recombinant DNA technologies, obviously gives reliable information about structure–function relationships. As we experienced in the 1950s and 1960s with many enzymes whose structures were later solved, X-ray crystallographic studies have not led us to correct our 3D models obtained by classic approaches. Rather, our

models have been confirmed and more important details have been added.

And the power of yet another method is confirmed by studies on LGCs: homology modelling. The agonist-binding domain of the glutamate receptor has a low-level sequence identity to various soluble bacterial periplasmic substrate-binding proteins for which high-resolution structures are available.^[50] These were used as a template to calculate the structure of the glutamate-receptor-binding domain, and to propose 3D features of the agonist-binding site. Now, with the first high-resolution structure of a glutamate-receptor binding domain in hand,^[37] we can see that despite the low degree of sequence similarity this approach yielded very reasonable results,^[51] and therefore we can be confident that the predictions made for other proteins also are essentially correct.

What is the use of such detailed knowledge? More than 50% of all synapses in our brain use glutamate as a transmitter. GluRs participate in brain development, and they are implicated in many neuropathies, ranging from epilepsy and strokes to Alzheimer's, Huntington's, and Parkinson's diseases. The treatment of these diseases requires a knowledge of the exact steric, chemical, and physical properties of the ligand-binding site to facilitate the design of drugs that target the glutamate receptors.

4.2. The Nicotinic Acetylcholine Receptors (nAChR)

4.2.1. General Features of the Family

The second LGC to be discussed herein is the nicotinic acetylcholine receptor (nAChR) as the best-characterized LGC and a prototypical structure for the class of 4TM receptors. Because of their homology, the other members of this superfamily are likely to share its structural features, in particular its transmembrane topology. A comprehensive collection of sequences and references regarding this super-

family is found at the web-site <http://www.pasteur.fr/LGIC/LGIC.html> (Table 2).^[52]

nAChRs are found mainly in postsynaptic membranes of central nervous system synapses and of the neuromuscular endplate. The physiological signal to which nAChR responds is the neurotransmitter acetylcholine, but it is also activated by nicotine, one of the most widely consumed drugs.

The nAChR ion channel is cation selective (other members of this superfamily such as the glycine receptor have anion-selective channels). In the case of the peripheral receptors, it is selective for Na⁺, K⁺, and, to a much lesser extent, for Ca²⁺, whereas some of the neuronal nAChR ion channels are mainly calcium channels. Receptor activation leads to depolarization of the plasma membrane; in neuronal cells, Ca²⁺ influx triggers Ca²⁺-mediated signalling processes.

No structure at atomic resolution is available for any of the receptors of this superfamily. For the nAChR, a three-dimensional picture is emerging from combined electron-microscopic, chemical-labeling, mutagenesis, and patch-clamp investigations (for a detailed review see ref. [53]). This structural information has been accumulated for the peripheral receptors, since nature has provided an extremely rich source in the form of the electric organs of electric rays and eels, from which the nAChRs can be easily purified in large amounts. The nAChR from the electric ray *Torpedo*, which serves as a model for the muscle-type receptors of higher vertebrates, has a molecular mass of about 290 kDa, about 20 kDa of which are glycosyl residues. It is a heteropentameric complex, composed of four different polypeptide chains with a stoichiometry of $\alpha_2\beta\gamma\delta$. The five subunits are arranged radially around the central ion channel in the order α_H - γ - α_L - δ - β in a clockwise orientation, when viewed from the synaptic cleft (see Figure 10); H and L designate the high- and low-affinity binding sites of the two α subunits, respectively—because of their different neighbours, the two α -subunits differ in their binding properties.^[54]

The other receptors of the superfamily presumably exist also as pentamers; this was directly demonstrated for

Table 2. Selected internet resources for navigating the field of ligand-gated ion channels.^[a]

Keyword	Short description	Internet address
General information on ion channels	A gateway to all the resources containing relevant information on ion channels	http://phy025.lubb.ttuhs.edu/Neely/ionchann.htm
General information on ion channels	A resource page for the ion channel community with many interesting links to ion-channel-related sites	http://members.nbci.com/IonChannel/ion.htm
Ligand-Gated Ion Channel (LGIC) Sequence Database (Le Novère and Changeux, 1999)	A well-maintained database containing the complete set (more than 300) of known nucleic-acid and amino-acid sequences of extracellularly activated transmitter-gated ion channels; sequence alignments and phylogenetic trees	http://www.pasteur.fr/LGIC/LGIC.html
GABA receptor database	GABAagent: a compilation of data on GABA receptors	http://gaba.ust.hk/Agent.html
Channel structure	A review of the structure of the KcsA channel with many figures of the pore	http://members.nbci.com/IonChannel/KcsA.htm
Permeation models	Different models on ion selectivity and conductance	http://www.ugrad.cs.jhu.edu/~imran/kcsa/
Interview with Roderick MacKinnon who solved the first channel structure (Doyle et al., 1988)	Mini-lecture on the fascinating intricacies of ion channel structures and the hard labor necessary to identify such a structure	http://www.sciam.com/explorations/2000/050100MacKinnon/index.html
Ion Channels and Disease	Highlights the molecular background of diseases linked to ion channels	http://www.neuro.wustl.edu/neuromuscular/mother/chan.html

[a] A more comprehensive list, including more general resources, is found in ref. [109].

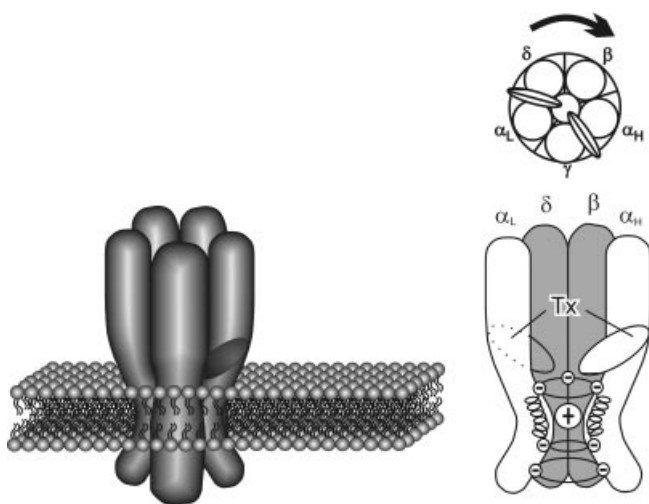


Figure 10. The interface gorge model of the nAChR. This figure summarizes our knowledge of the structure of the nAChR. Left: global layout of the receptor in its membrane surrounding; Right (top): arrangement of the receptor subunits around the central pore, viewed from the synaptic cleft. Right (bottom): the longitudinal section shows that the ligand-binding site spans the receptor wall. The ion channel is formed by transmembrane helices; the channel's gate is a restriction towards the cytoplasmic side of the membrane. Tx refers to α -neurotoxin, the peptide toxin from poisonous snakes, which was used to determine the ligand-binding topography.^[87] The ellipsoid represents the toxin-binding site; since the α -neurotoxins are competitive antagonists, it is also thought to form (at least partially) the agonist-binding site.

GABA_AR and 5HT₃R by using electron microscopy,^[55, 56] and indirectly for the GlyR by using biochemical techniques.^[57, 58] A heteromeric assembly of different subunits is the rule and, as explained above, is the basis for receptor diversity, but a homomeric assembly has also been described, for example, for the 5HT₃R and for certain neuronal nicotinic and GABA_AR subtypes.^[59, 60]

The four nAChR subunits display homologous sequences with 30 to 40% identity of amino-acid residues. Hydropathy plots of the primary structures reveal that they each contain four hydrophobic stretches, which are thought to form membrane-spanning segments (M1–M4). All members of the family share this pattern. They all have extracellular N and C termini, a large extracellular glycosylated N-terminal domain (about 200 residues long), and a long cytosolic connecting loop of 100 to 150 residues between the membrane passages M3 and M4 which contains several phosphorylation sites.

A further characteristic motif of this receptor superfamily is a loop formed by a pair of cysteine residues at a 15-residue spacing in the N-terminal domain (Figure 3). The two cysteine residues are connected by a disulphide bridge,^[61] and the residues between them are highly conserved. This Cys loop, for which a β -folded amphipathic structure is predicted,^[62] occurs independently of the ion selectivity and ligand-binding properties of the receptor at a fixed position relative to the first membrane passage, and might play an important role as a core in protein folding. As this Cys loop forms a typical signature for this superfamily, it has been proposed to call this superfamily “Cys loop receptors”.^[63]

The overall dimension of the pentameric nAChR complex, as deduced from electron micrographs (obtained by using cryo-electron microscopy of tubular crystals grown from membranes isolated from the *Torpedo* electric organ) is about 12.5×8 nm.^[64] One can estimate that about 35% of the protein is immersed in the membrane and about 50% is extracellular.

4.2.2. The Ion Channel—Selectivity Filter and Gate

In the electron micrographs discussed in the previous section, one also readily discerns a central hole in the receptor “doughnut”, which is about 20 to 25 Å wide.^[65] This structure is interpreted as the entrance to the ion channel. Thus, very much like the potassium channel discussed above, the nAChR channel appears to have a wide water-filled extramembrane vestibule, which is followed by a funnel-shaped portion inside the membrane (Figure 10). Its diameter is 11.5 Å for the middle part (determined by the size of channel blockers used to label specific residues of the channel wall^[66]) and about 7 Å in the open state at the narrowest part towards the cytosolic end (predicted by computer modelling^[67]). This value corresponds well to the diameter of the largest permeating cation (6.4 Å).^[68] At the cytoplasmic end, the pore widens again to about 15 Å. Electron micrographs show that underneath the pore, a basketlike structure formed by the cytoplasmic loops between M3 and M4 protrudes about 40 Å from the cytoplasmic membrane surface. It was suggested that ions are prevented by this structure from exiting directly into the cytoplasm; instead they must pass through two narrow transverse openings.^[69]

The wall of the ion channel is formed by the second transmembrane segment (M2) of each receptor subunit.^[66] This was concluded mainly from affinity-labeling studies of substances that directly occlude the ion channel (channel blockers);^[66, 70] this finding was confirmed by mutational analysis.^[71, 72] From the pattern of labeled amino-acid residues, we know that this segment adopts, at least partially, an α -helix conformation, a conclusion largely confirmed by chemical accessibility studies.^[73] Parts of the first transmembrane segment M1 also contribute at least transiently to the channel lining in the wider upper (extracellular) part.^[74]

The five homologous subunits each contribute the corresponding segment for the construction of the ion channel. An interesting stratification of the channel wall results from this construction principle (Figure 11): rings of amino-acid side chains with similar physical properties are formed which determine the characteristic features of the channel—most conspicuously three negatively charged rings (Asp/Glu), three hydrophobic rings (Leu/Val), and two rings of polar residues (Ser/Thr).^[75] The ion conductance properties, for example, are explained by three rings of negatively charged amino-acid side chains.^[76] These rings are located at the cytoplasmic N-terminal and the extracellular C-terminal end of the channel and midway between the two. The intermediate ring, in which glutamate side chains protrude into the channel lumen, forms the constriction that determines which ions can pass. Gating—the opening and closing of the permeation pathway—could be brought about by moving these side

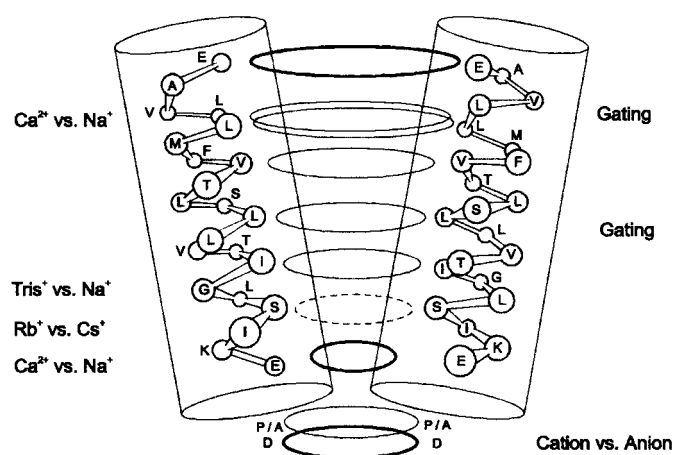


Figure 11. Stratification model of the ion channel of the nAChR. Homologous side chains of the five receptor subunits form rings, which determine the properties of the channel (ion selectivity and gating) as elucidated by mutagenesis experiments (simplified from ref. [75]). Depicted are the M2 sequences from the neuronal $\alpha 7$ subunits. (Tris = tris(hydroxymethyl)aminomethane)

chains out of the channel lumen,^[67] perhaps by a similar “rigid-body movement” of the helices as was postulated for the gating of the voltage-dependent potassium channels.^[48] Other experiments showed that one of the hydrophobic rings formed by a conserved leucine residue at the approximate midpoint of the M2 transmembrane segment (Leu9') contributes to channel gating. It was suggested that these leucine residues from the different subunits participate in a symmetric structural transition between the closed and open states.^[77]

The role of the selectivity filter, in which large ions are selected according to their dehydrated size, was assigned more specifically to the amino-acid residue α T244 that is situated one helix turn above this intermediate ring.^[78] The segment α 238–242 just below this ring was shown to contribute directly to the charge selectivity filter.^[79] This segment is at the cytoplasmic border of the M2 membrane span and is predicted to adopt a coiled conformation, reminiscent of the P loop described above.^[80]

By using the substituted-cysteine accessibility method^[81]—the residues are individually mutated to cysteine, the accessibility of which is then assessed with small sulfhydryl-specific reagents—the gate was located in the same region (between α G240 and α T244) in the vicinity of the intermediate ring.^[82] Thus the gate and selectivity filter seem to be intimately linked.

The construction principle defined in this “helix-M2 model”^[66] (Figure 10) for the cation-selective nAChR channel is also thought to be valid for the other members of this protein superfamily, for example, the anion-selective channel of the GlyR. A comparison between the amino acid residues at the critical positions of these two channels demonstrates that the conversion of the cation-selective nAChR channel into an anion-selective channel can be brought about by introducing mutations at only three positions.^[83] By the inverse mutations, the anion-selective channel of the GlyR can be transformed into a cation-selective channel.^[84] It is mainly the substitution of the intermediate negatively charged

ring by a neutral ring that is relevant for cation versus anion discrimination.^[79] This type of manipulation proves that our concept on how the ion selectivity of the AChR ion channel is regulated is essentially correct.

4.2.3. The Ligand-Binding Site: An Intersubunit Gorge

Now let's have a closer look at the ligand-binding site of the nAChR (“L” in Figure 1). In contrast to the glutamate receptors reviewed in Section 4.1.2, the extracellular domain of the nAChR involved in ligand binding has not yet been crystallized and no high-resolution X-ray structure is available. The binding site itself, however, has been characterized in some detail by using molecular biology and biochemical methods so that a spatial model of the binding pocket was obtained. The region that contains amino acids 190–200 on the two identical α -subunits as well as several functionally important aromatic residues was shown to contribute to the binding site by affinity labeling using various agonists and antagonists. However, there is more to the story: some amino-acid residues of the α -subunits outside this region are also labeled, and even portions of the neighboring subunits (that is, the γ - and the δ -subunits) located close to the interfaces contribute to the binding site.^[85] From these findings, a model of the α -subunit binding site for low-molecular-mass ligands was proposed (Figure 12) which states that the binding site is

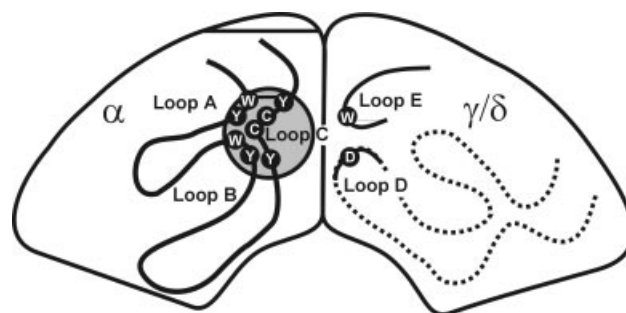


Figure 12. Model of the nAChR agonist-binding site. Three loops of the α -subunit contribute to the binding site, which is located at the subunit interface. Note that additional loops extend from the neighboring subunits.

not confined to a contiguous stretch of the primary structure of the ligand-binding α -subunit, but is built from three noncontiguous loops of the α -subunit.^[86] Additional loops come in from the neighboring subunits. This finding also provides a rational basis for the nonequivalence of the two ligand-binding sites for certain ligands such as d-tubocurarine, which has a high-affinity site at the α/γ interface and a low affinity site at the α/δ interface.

Studies of snake-venom α -neurotoxins as affinity reagents led to the hypothesis that the toxin binds in relative proximity (1.5 nm or closer) to the membrane layer close to the beginning of the funnel structure formed by the M2 spans.^[87] It was shown experimentally that the toxin gains access to this site through a gorge spanning the wall of the ring-shaped receptor, which contains the aromatic residues of the ligand-binding site (“aromatic interface gorge”; Figure 10).^[53, 88] In contrast, tunnels about 10 to 15 Å long which open only to the

vestibule and are framed by twisted β -sheet strands were resolved recently in the extracellular portion of the α -subunits by using electron microscopy.^[69] They connect cavities (interpreted as the acetylcholine-binding sites) to the water-filled vestibule of the channel and are believed to represent the primary access route of agonists to the binding site.

Such a design would parallel the situation in the ACh-degrading enzyme acetylcholinesterase in which the acetylcholine-binding site is located at the bottom of a 20-Å deep aromatic gorge.^[89] Perhaps the analogy with the situation in AChE can even be extended to the binding mode: In both cases binding of the cholinium moiety is achieved by cation- π interaction, in which the face of an aromatic ring provides a region of negative electrostatic potential that can bind cations with considerable strength.^[90] With the ACh esterase it has been shown by site-directed affinity labelling that the binding side chain is the tryptophan residue Trp84.^[91] A combination of theoretical chemistry with molecular biology experiments suggest that α Trp149 is the primary cation- π binding site for AChR.^[92]

Thus the nAChR's ligand-binding site appears to be an intersubunit gorge close to the membrane surface and also close to the channel entrance, made up of at least three different loops of the α -subunits containing numerous aromatic residues as well as negatively charged portions of the neighboring γ - and the δ -subunits.

Operationally, receptors can be described as a triune entity, composed of 1) a signal-recognition moiety (receptor, "R"), 2) an effector function ("E"), and 3) a transducing component ("T"), which couples R to E.^[53] In the nAChR, the sites for agonist binding ("R"), conformational signal transfer to the ion channel ("T"), and the ion channel itself ("E") are confined within a rather restricted area of only about 2 nm which would form a sort of "active center".^[53] The transducing mechanism in this case is the allosteric transition that occurs upon neurotransmitter binding.

Despite this spatial integration, it seems that the extracellular domain and the channel fold independently from each other, since a chimeric molecule could be constructed from two different receptor types (neuronal α 7nAChR and serotonin 5HT₃R) which combined the binding properties of one and the channel properties of the other partner.^[93]

Interestingly, the high-resolution structure (3.5 Å) of a mechanosensitive ion channel from *Mycobacterium tuberculosis* reveals several features that are very similar to the nAChR (Figure 13).^[94] as in the case of the nAChR, the structure is pentameric and a wide water-filled vestibule of about 18 Å in diameter leads from the extracellular side into a pore, which narrows towards the cytosolic side. In both channels, the walls are formed by helices lined with polar residues such as threonine and serine. In both cases, hydrophobic residues are present at the constriction that may act as the channel gate. The structure formed intracellularly in the mechanosensitive channel is reminiscent of the "basket" observed in electron micrographs of the nAChR.^[69]

Despite our detailed knowledge of the nAChR, several key questions remain open and probably will be answered only when a high-resolution structure becomes available. So the

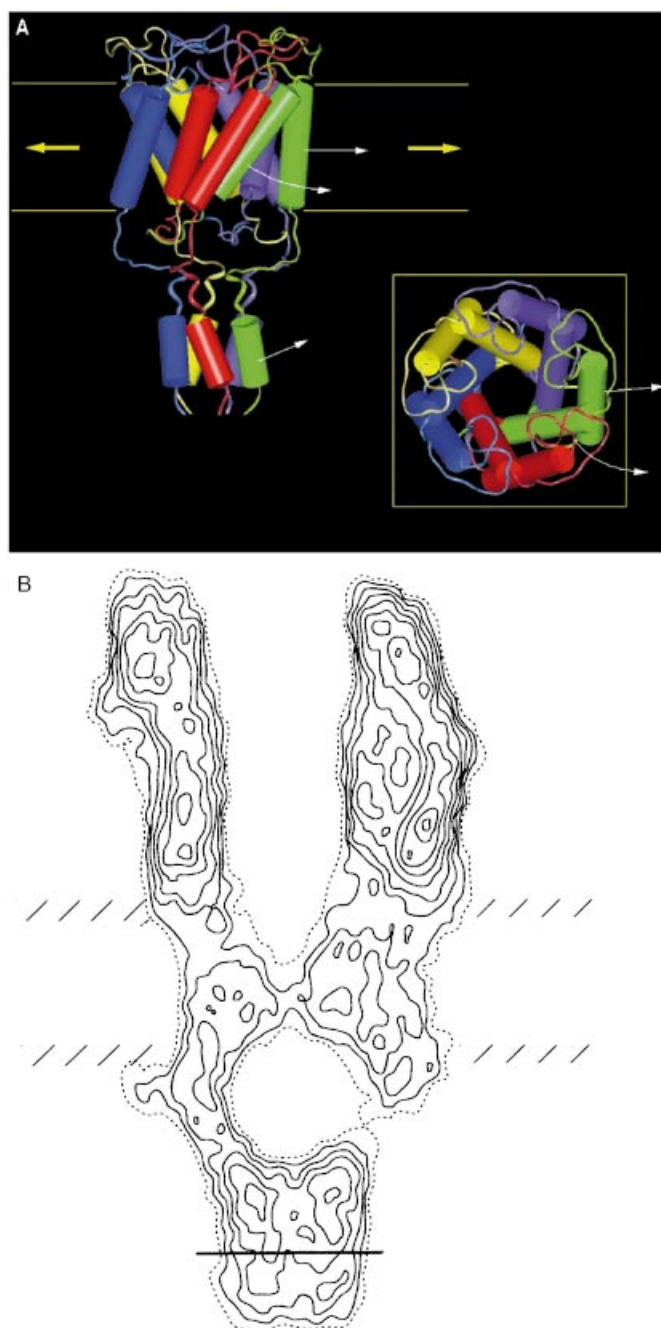


Figure 13. Similarities in the overall structures of a mechanosensitive channel (A) and nAChR (B). A) X-ray structure of a mechanosensitive ion channel from *Mycobacterium tuberculosis*.^[94] B) Electron micrograph (4.6-Å resolution) of the nAChR from *Torpedo californica*.^[69] Both pentameric structures have a wide water-filled vestibule that leads into the channel pore and an intracellular basket-like structure underneath the channel exit. Courtesy of Douglas Rees, reproduced with permission of *Science* and the author(A), and courtesy of Nigel Unwin (B).

precise coupling mechanism by which the detection of a chemical signal (agonist binding) is translated into the opening of the ion permeation pathway (channel opening) remains to be described in detail. Furthermore, the pharmacologically fundamental functional difference between agonists and antagonists, both of which bind to the same site, remains elusive.

4.3. The Purinoceptor Family P2X

The last class of ligand-gated ion channels to be discussed herein are the P2X receptors gated by extracellular ATP (as opposed to P2Y receptors which belong to the G-protein-coupled “metabotropic” receptors mentioned in the introduction). They are cation-selective channels that open in the millisecond range upon binding of extracellular ATP. To date, seven P2X isoforms, referred to as P2X_{1–7}, have been cloned (reviewed in ref. [95]). The deduced proteins display sequence similarity between themselves (38 to 48% amino-acid identity when pairs are compared), but no homology with any of the other ligand-gated ion channels.^[96] Although no high-resolution structure is available, it is clear that some of their structural features are fundamentally different from the two classes of LGCs discussed above. Most importantly, they have a remarkably different predicted transmembrane arrangement: each subunit—about 400 amino acids in length and with both termini inside the cell—is thought to contain only two membrane-spanning segments (M1 and M2), connected by a large (270 residues) glycosylated extracellular loop. The extracellular domain contains ten cysteine residues, which may form five intrachain disulfide bonds.^[97] These are believed to be important for the overall structure, since they are perfectly conserved in all family members.^[96] The precise structure of the agonist-binding site is still not known. Mutational studies showed that a ATP-binding pocket that contains some positively charged residues forms close to the channel vestibule.^[98, 99]

P2X receptors are nonselectively permeable to cations, including Ca²⁺; the effective pore diameter is somewhat larger than that of the nAChR.^[100] Studies on the accessibility of engineered cysteine residues show that the M2 segment contributes to the ion permeation pathway.^[101] The results imply that at least part of the pore would be in an extended rather than in a helical conformation, but how exactly the pore is organized remains unclear. A conserved glycine residue (Gly 342 of P2X₂) may be part of the gate.^[102]

An intriguing feature observed in some ion channels is their dynamic selectivity filter (discussed in ref. [103]). For several P2X subtypes, a progressive dilation of the pore is observed upon prolonged activation by ATP so that large molecules can permeate.^[104] How this dynamic behavior is brought about and whether and how ionotropic receptors use this mechanism to alter neuronal function remains to be shown. Another interesting aspect of P2X channels is that some of them seem to interact functionally with the structurally unrelated nAChRs, thus establishing a cross-talk between differently gated channels.^[105]

Similarly, the oligomeric organization of P2X receptors seems to deviate from the architecture observed in GluR and AChR. The precise quaternary structure of functionally active P2X receptors remains to be established. Although they share their transmembrane topology with a variety of other ion channels such as the epithelial Na⁺ channels or the inwardly rectifying K⁺ channels, no common oligomeric structure can be deduced.^[106] There are hints that some of these receptors share a similar architecture, with trimers as the common denominator. For the P2X receptors, electrophoresis studies

and cross-linking experiments indicate that they occur as homotrimers, or at least that trimeric complexes of identical subunits constitute an essential element.^[5] As with the other LGC classes, hetero-oligomeric assembly is possible and might underlie functional diversity.^[107]

5. Conclusions and Overview

Thanks to the availability of high-resolution X-ray structures of voltage-gated ion channels and receptor domains, we have learnt a lot about the structural basis of the gating and selectivity in LGCs. Clearly, many questions are still unanswered and can only be answered when the Å-resolution structures of LGCs become available. Recently, the first structure of a G-protein-coupled receptor (rhodopsin) was solved.^[108] This indicates that the techniques for crystallizing membrane proteins have reached a stage where crystallization of LGCs should also become feasible in the near future.

Addendum

After the submission of this manuscript, two important relevant articles were published:^[110] An acetylcholine-binding protein (AChBP) from the mollusc *Lymnaea stagnalis* has been shown to have significant structural homology with the extracellular ligand-binding domain of the neuronal nAChR. Since AChBP is a water-soluble protein, it has been crystallized. The high-resolution (2.7 Å) X-ray structure can be seen as a model of the binding domain of the LGC nAChR (Figure 14).

We thank Henning Otto and Gabi Bixel for critically reading this manuscript. Work from the authors' laboratory discussed herein was supported by the Deutsche Forschungsgemeinschaft (SFB 449) and the Fonds der Chemischen Industrie.

Received: November 27, 2000 [A 438]

- [1] H. M. Lam, J. Chiu, M. H. Hsieh, L. Meisel, I. C. Oliveira, M. Shin, G. Coruzzi, *Nature* **1998**, *396*, 125–126.
- [2] J. Chiu, R. DeSalle, H. M. Lam, L. Meisel, G. Coruzzi, *Mol. Biol. Evol.* **1999**, *16*, 826–838.
- [3] F. Hucho, J.-P. Changeux, *FEBS Lett.* **1973**, *38*, 11–15.
- [4] B. Laube, J. Kuhse, H. Betz, *J. Neurosci.* **1998**, *18*, 2954–2961.
- [5] A. Nicke, H. G. Baumert, J. Rettinger, A. Eichele, G. Lambrecht, E. Mutschler, G. Schmalzing, *EMBO J.* **1998**, *17*, 3016–3028.
- [6] K. Kiselyov, G. A. Mignery, M. X. Zhu, S. Muallem, *Mol. Cell* **1999**, *4*, 423–429.
- [7] G. Boulay, D. M. Brown, N. Qin, M. Jiang, A. Dietrich, M. X. Zhu, Z. Chen, M. Birnbaumer, K. Mikoshiba, L. Birnbaumer, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14955–14960.
- [8] M. J. Caterina, M. A. Schumacher, M. Tominaga, T. A. Rosen, J. D. Levine, D. Julius, *Nature* **1997**, *389*, 816–824.
- [9] M. A. Raftery, M. W. Hunkapiller, C. D. Strader, L. E. Hood, *Science* **1980**, *208*, 1454–1456.
- [10] W. Hevers, H. Lüddens, *Mol. Neurobiol.* **1998**, *18*, 35–86.
- [11] B. Sommer, M. Kohler, R. Sprengel, P. H. Seeburg, *Cell* **1991**, *67*, 11–19.
- [12] P. H. Seeburg, *J. Neurochem.* **1996**, *66*, 1–5.
- [13] F. Hucho, *Angew. Chem.* **1994**, *106*, 23–36; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 39–50.

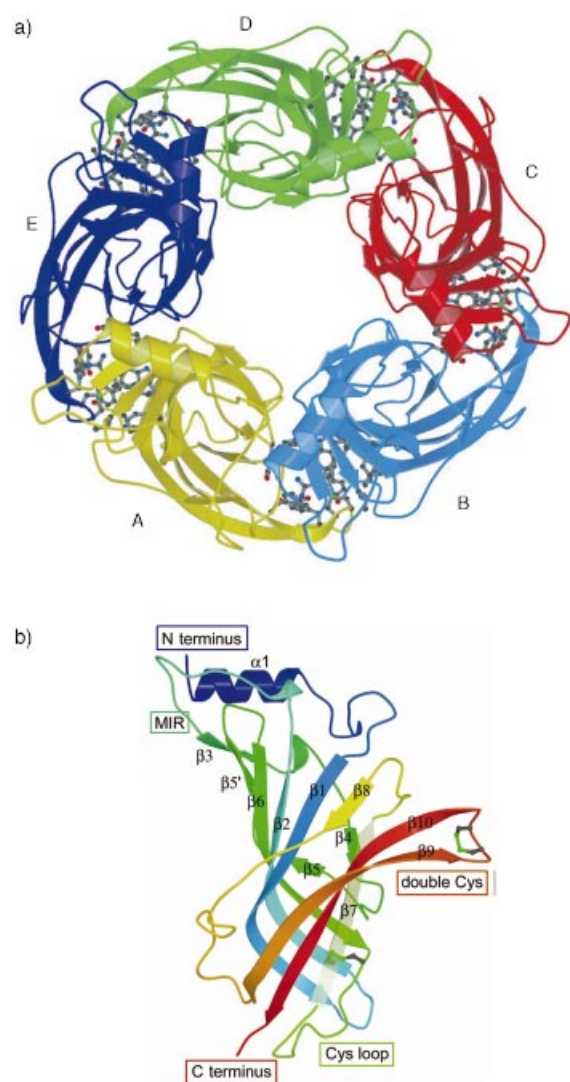


Figure 14. The pentameric structure of AChBP. a) In this representation, each promoter has a different color; subunits are labelled anticlockwise. The ligand-binding sites at the subunit interfaces are shown as ball-and-stick representations. b) Overview of the AChBP promoter structure as viewed from outside the pentameric ring. Rainbow representation colored as a gradient, from blue (N terminus) to red (C terminus); disulfide bridges are shown as green ball-and-stick representations. MIR stands for main immunogenic region. For more details on the Cys loop and double Cys, see text. In a complete LGIC, the N terminus would point towards the synaptic cleft and the C terminus would enter the membrane at the bottom, contributing to the first transmembrane segment. (Modified from ref. [110], with kind permission from the authors.)

- [14] S. J. Edelstein, J.-P. Changeux, *Adv. Protein Chem.* **1998**, *51*, 121–184.
- [15] A. L. Mammen, S. Kamboj, R. L. Haganir, *Methods Enzymol.* **1999**, *294*, 353–370.
- [16] S. L. Swope, Z. Qu, R. L. Haganir, *Ann. N. Y. Acad. Sci.* **1995**, *757*, 197–214.
- [17] J. Kirsch, D. Langosch, P. Prior, U. Z. Littauer, B. Schmitt, H. Betz, *J. Biol. Chem.* **1991**, *266*, 22242–22245.
- [18] H. Wang, F. K. Bedford, N. J. Brandon, S. J. Moss, R. W. Olsen, *Nature* **1999**, *397*, 69–72.
- [19] H.-C. Kornau, L. T. Schenker, M. B. Kennedy, P. H. Seeburg, *Science* **1995**, *269*, 1737–1740.
- [20] H. Dong, R. J. O'Brien, E. T. Fung, A. A. Lanahan, P. F. Worley, R. L. Haganir, *Nature* **1997**, *386*, 279–284.
- [21] M. Sheng, D. T. Pak, *Annu. Rev. Physiol.* **2000**, *62*, 755–78.
- [22] H. Husi, M. A. Ward, J. S. Choudhary, W. P. Blackstock, S. G. Grant, *Nat. Neurosci.* **2000**, *3*, 661–669.
- [23] W. Hoch, *Eur. J. Biochem.* **1999**, *265*, 1–10.
- [24] T. Green, S. F. Heinemann, J. F. Gusella, *Neuron* **1999**, *20*, 427–444.
- [25] A. Vincent, C. Newland, R. Croxson, D. Beeson, *Trends Neurosci.* **1997**, *20*, 15–22.
- [26] C. Beck, B. Moulard, O. Steinlein, M. Guipponi, L. Vallee, P. Montpied, M. Blady-Moulner, A. Malafosse, *Neurobiol. Dis.* **1994**, *1*, 95–99.
- [27] O. K. Steinlein, J. C. Mulley, P. Propping, R. H. Wallace, H. A. Phillips, G. R. Sutherland, I. E. Scheffer, S. F. Berkovic, *Nat. Genet.* **1995**, *11*, 201–203.
- [28] S. G. Ryan, S. L. Sherman, J. C. Terry, R. S. Sparkes, M. C. Torres, R. W. Mackey, *Ann. Neurol.* **1992**, *31*, 663–668.
- [29] A. Vincent, D. Beeson, B. Lang, *Eur. J. Biochem.* **2000**, *267*, 6717–6728.
- [30] E. K. Michaelis, *Prog. Neurobiol.* **1997**, *54*, 369–415.
- [31] C. F. Bigge, *Curr. Opin. Chem. Biol.* **1999**, *3*, 441–447.
- [32] R. Dingleline, K. Borges, D. Bowie, S. F. Traynelis, *Pharmacol. Rev.* **1999**, *51*, 7–61.
- [33] D. A. Doyle, J. Morais Cabral, R. A. Pfuertner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. MacKinnon, *Science* **1998**, *280*, 69–77; the atomic coordinates are deposited at the Protein Data Bank at <http://www.rcsb.org/pdb/> as entry 1BL8.
- [34] G. Q. Chen, C. Cui, M. L. Mayer, E. Gouaux, *Nature* **1999**, *402*, 817–821.
- [35] R. I. Hume, R. Dingleline, S. F. Heinemann, *Science* **1991**, *253*, 1028–1031.
- [36] L. Simpson, R. B. Emeson, *Annu. Rev. Neurosci.* **1996**, *19*, 27–52.
- [37] N. Armstrong, Y. Sun, G. Q. Chen, E. Gouaux, *Nature* **1998**, *395*, 913–917; the atomic coordinates are deposited at the Protein Data Bank at <http://www.rcsb.org/pdb/> as entry 1GR2.
- [38] Y. Stern-Bach, B. Bettler, M. Hartley, P. O. Sheppard, P. J. O'Hara, S. F. Heinemann, *Neuron* **1994**, *13*, 1345–1357.
- [39] A. Kuusinen, M. Arvola, K. Keinänen, *EMBO J.* **1995**, *14*, 6327–6332.
- [40] G. Q. Chen, E. Gouaux, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13431–13436.
- [41] G. Q. Chen, Y. Sun, R. Jin, E. Gouaux, *Protein Sci.* **1998**, *7*, 2623–2630.
- [42] Y. J. Sun, J. Rose, B. C. Wang, C. D. Hsiao, *J. Mol. Biol.* **1998**, *278*, 219–229.
- [43] Y. Paas, M. Eisenstein, F. Medevielle, V. I. Teichberg, A. Devillers-Thiery, *Neuron* **1996**, *17*, 979–990; the 3D model can be downloaded from the LGIC database (see Table 1).
- [44] K. Keinänen, M. Arvola, A. Kuusinen, M. Johnson, *Biochem. Soc. Trans.* **1997**, *25*, 835–838.
- [45] B. Laube, H. Hirai, M. Sturgess, H. Betz, J. Kuhse, *Neuron* **1997**, *18*, 493–503.
- [46] N. Armstrong, E. Gouaux, *Neuron* **2000**, *28*, 165–181.
- [47] a) T. Baukrowitz, *Eur. J. Biochem.* **2000**, *267*, 5823–5848; b) D. Oliver, T. Baukrowitz, B. Fakler, *Eur. J. Biochem.* **2000**, *267*, 5824–5829; c) M. D. Mark, S. Herlitze, *Eur. J. Biochem.* **2000**, *267*, 5830–5836; d) U. Schulte, B. Fakler, *Eur. J. Biochem.* **2000**, *267*, 5837–5841; e) T. Baukrowitz, B. Fakler, *Eur. J. Biochem.* **2000**, *267*, 5842–5848.
- [48] E. Perozo, D. M. Cortes, L. G. Cuello, *Science* **1999**, *285*, 73–78.
- [49] B. A. Yi, L. Y. Jan, *Neuron* **2000**, *27*, 423–425.
- [50] P. J. O'Hara, P. O. Sheppard, H. Thogersen, D. Venezia, B. A. Haldeman, V. McGrane, K. M. Houamed, C. Thomsen, T. L. Gilbert, E. R. Mulvihill, *Neuron* **1993**, *11*, 41–52.
- [51] Y. Paas, A. Devillers-Thiery, V. I. Teichberg, J.-P. Changeux, M. Eisenstein, *Trends Pharmacol. Sci.* **2000**, *21*, 87–92.
- [52] N. Le Novère, J.-P. Changeux, *Nucleic Acids Res.* **1999**, *27*, 340–342.
- [53] F. Hucho, V. I. Tsetlin, J. Machold, *Eur. J. Biochem.* **1996**, *239*, 539–557.
- [54] J. Machold, C. Weise, Yu. Utkin, V. Tsetlin, F. Hucho, *Eur. J. Biochem.* **1995**, *234*, 427–430.
- [55] N. Nayeem, T. P. Green, I. L. Nartin, E. A. Barnard, *J. Neurochem.* **1994**, *62*, 815–818.
- [56] F. G. Boess, R. Beroukhir, I. L. Martin, *J. Neurochem.* **1995**, *64*, 1401–1405.

- [57] D. Langosch, L. Thomas, H. Betz, *Proc. Natl. Acad. Sci. USA* **1988**, 85, 7394–7398.
- [58] N. Griffon, C. Buttner, A. Nicke, J. Kuhse, G. Schmalzing, H. Betz, *EMBO J.* **1999**, 18, 4711–4721.
- [59] S. Couturier, D. Bertrand, J. M. Matter, M. C. Hernandez, S. Bertrand, N. Millar, S. Valera, T. Barkas, M. Ballivet, *Neuron* **1990**, 5, 847–856.
- [60] D. B. Pritchett, H. Sontheimer, C. M. Gorman, H. Kettenmann, P. H. Seeburg, P. R. Schofield, *Science* **1988**, 242, 1306–1308.
- [61] P. N. Kao, A. Karlin, *J. Biol. Chem.* **1986**, 261, 8085–8088.
- [62] V. B. Cockcroft, D. J. Osguthorpe, E. A. Barnard, G. C. Lunt, *Proteins* **1990**, 8, 386–397.
- [63] P. P. A. Humphrey, E. A. Barnard, *Pharmacol. Rev.* **1998**, 50, 271–277.
- [64] N. Unwin, *Nature* **1995**, 373, 37–43.
- [65] N. Unwin, *J. Mol. Biol.* **1993**, 229, 1101–1124.
- [66] F. Hucho, W. Oberthür, F. Lottspeich, *FEBS Lett.* **1986**, 205, 137–142.
- [67] F. Hucho, R. Hilgenfeld, *FEBS Lett.* **1989**, 257, 17–23.
- [68] L. Y. Huang, W. A. Catterall, G. Ehrenstein, *J. Gen. Physiol.* **1978**, 71, 397–410.
- [69] A. Miyazawa, Y. Fujiyoshi, M. Stowell, N. Unwin, *J. Mol. Biol.* **1999**, 288, 765–786.
- [70] J. Giraudat, M. Dennis, T. Heidmann, J.-Y. Chang, J.-P. Changeux, *Proc. Natl. Acad. Sci. USA* **1986**, 83, 2719–2723.
- [71] K. Imoto, C. Methfessel, B. Sakmann, M. Mishina, Y. Mori, T. Konno, K. Fukuda, M. Kurasaki, H. Bujo, Y. Fujita, S. Numa, *Nature* **1986**, 324, 670–674.
- [72] R. J. Leonard, C. G. Labarca, P. Charnet, N. Davidson, H. A. Lester, *Science* **1998**, 242, 1578–1581.
- [73] M. H. Akabas, C. Kaufmann, P. Archdeacon, A. Karlin, *Neuron* **1994**, 13, 919–927.
- [74] M. H. Akabas, A. Karlin, *Biochemistry* **1995**, 34, 12496–12500.
- [75] D. Bertrand, J.-L. Galzi, A. Devillers-Thiéry, S. Bertrand, J.-P. Changeux, *Curr. Opin. Cell Biol.* **1993**, 5, 688–693.
- [76] K. Imoto, C. Busch, B. Sakmann, M. Mishina, T. Konno, J. Nakai, H. Bujo, J. Mori, K. Fukuda, S. Numa, *Nature* **1988**, 335, 645–648.
- [77] C. Labarca, M. W. Nowak, H. Zhang, L. Tang, P. Deshpande, H. A. Lester, *Nature* **1995**, 376, 514–516.
- [78] A. Villarroel, B. Sakmann, *Biophys. J.* **1992**, 62, 196–208.
- [79] P.-J. Corringer, S. Bertrand, J.-L. Galzi, A. Devillers-Thiéry, J.-P. Changeux, D. Bertrand, *Neuron* **1999**, 22, 831–843.
- [80] N. Le Novère, P.-J. Corringer, J.-P. Changeux, *Biophys. J.* **1999**, 76, 2329–2345.
- [81] A. Karlin, M. H. Akabas, *Methods Enzymol.* **1998**, 293, 123–145.
- [82] G. G. Wilson, A. Karlin, *Neuron* **1998**, 20, 1269–1281.
- [83] J.-L. Galzi, A. Devillers-Thiéry, N. Hussy, S. Bertrand, J.-P. Changeux, *Nature* **1992**, 359, 500–505.
- [84] A. Keramidas, A. J. Moorhouse, C. R. French, P. R. Schofield, P. H. Barry, *Biophys. J.* **2000**, 79, 247–259.
- [85] a) D. C. Chiara, J. B. Cohen, *Biophys. J.* **1992**, 61, A106; b) S. M. Sine, *Proc. Natl. Acad. Sci. USA* **1993**, 90, 9436–9440; c) C. Czajkowski, A. Karlin, *J. Biol. Chem.* **1995**, 270, 3160–3164; d) R. J. Prince, S. M. Sine, *J. Biol. Chem.* **1996**, 271, 25770–25777.
- [86] J.-L. Galzi, J.-P. Changeux, *Curr. Opin. Struct. Biol.* **1994**, 4, 554–565.
- [87] J. Machold, Yu. Utkin, D. Kirsch, R. Kaufmann, V. Tsetlin, F. Hucho, *Proc. Natl. Acad. Sci. USA* **1995**, 92, 7282–7286.
- [88] P. Saez-Briones, M. Krauss, M. Dreger, A. Herrmann, V. I. Tsetlin, F. Hucho, *Eur. J. Biochem.* **1999**, 265, 902–910.
- [89] J. L. Sussman, M. Harel, F. Frolow, C. Oefner, A. Goldman, L. Toker, I. Silman, *Science* **1991**, 253, 872–879.
- [90] J. C. Ma, D. A. Dougherty, *Chem. Rev.* **1997**, 97, 1303–1324.
- [91] C. Weise, H.-J. Kreienkamp, R. Raba, A. Pedak, A. Aaviksaar, F. Hucho, *EMBO J.* **1990**, 9, 3885–3888.
- [92] W. Zhong, J. P. Gullivan, Y. Zhang, L. Li, H. A. Lester, *Proc. Natl. Acad. Sci. USA* **1998**, 95, 12088–12093.
- [93] J.-L. Eiselé, S. Bertrand, J.-L. Galzi, A. Devillers-Thiéry, J.-P. Changeux, D. Bertrand, *Nature* **1993**, 366, 479–483.
- [94] G. Chang, R. H. Spencer, A. T. Lee, M. T. Barclay, D. C. Rees, *Science* **1998**, 282, 2220–2226.
- [95] R. A. North, E. A. Barnard, *Curr. Opin. Neurobiol.* **1997**, 7, 346–357.
- [96] R. A. North, *Ciba Found. Symp.* **1996**, 198, 91–105.
- [97] M. A. Hansen, J. A. Barden, V. J. Balcar, K. A. Keay, M. R. Bennett, *Biochem. Biophys. Res. Commun.* **1997**, 236, 670–675.
- [98] S. Ennion, S. Hagan, R. J. Evans, *J. Biol. Chem.* **2000**, 275, 29361–29367.
- [99] L. H. Jiang, F. Rassendren, A. Surprenant, R. A. North, *J. Biol. Chem.* **2000**, 275, 34190–34196.
- [100] R. J. Evans, C. Lewis, C. Virginio, K. Lundstrom, G. Buell, A. Surprenant, R. A. North, *J. Physiol.* **1996**, 497, 413–422.
- [101] F. Rassendren, G. Buell, A. Newbolt, R. A. North, A. Surprenant, *EMBO J.* **1997**, 16, 3446–3454.
- [102] T. M. Egan, W. R. Haines, M. M. Voigt, *J. Neurosci.* **1998**, 18, 2350–2359.
- [103] S. Khakh, H. A. Lester, *Neuron* **1999**, 23, 653–638.
- [104] a) A. Surprenant, F. Rassendren, E. Kawashima, R. A. North, G. Buell, *Science* **1996**, 272, 735–738; b) C. Virginio, A. MacKenzie, F. A. Rassendren, R. A. North, A. Surprenant, *Nat. Neurosci.* **1999**, 2, 315–321; c) B. S. Khakh, X. R. Bao, C. Labarca, H. A. Lester, *Nat. Neurosci.* **1999**, 2, 322–330.
- [105] B. S. Khakh, X. Zhou, J. Sydes, J. J. Galligan, H. A. Lester, *Nature* **2000**, 406, 405–410.
- [106] R. A. North, *Curr. Opin. Cell Biol.* **1996**, 8, 474–483.
- [107] G. E. Torres, T. M. Egan, M. M. Voigt, *J. Biol. Chem.* **1999**, 274, 6653–6659.
- [108] K. Palczewski, T. Kumasaka, T. Hori, C. A. Behnke, H. Motoshima, B. A. Fox, I. Le Trong, D. C. Teller, T. Okada, R. E. Stenkamp, M. Yamamoto, M. Miyano, *Science* **2000**, 289, 739–745.
- [109] J. Packer, E. Conley, N. Castle, D. Wray, C. January, L. Patmore, *Trends Pharmacol. Sci.* **2000**, 21, 327–329.
- [110] a) A. B. Smit, N. I. Syed, D. Schaap, J. van Minnen, J. Klumpermann, K. S. Kits, H. Lodder, R. C. van der Schors, R. van Elk, B. Sorgedrager, K. Brejc, T. K. Sixma, W. P. Geraerts, *Nature* **2001**, 411, 261–268; b) K. Brejc, W. J. van Dijk, R. V. Klaassen, M. Schuurmanns, J. van der Oost, A. B. Smit, T. K. Sixma, *Nature* **2001**, 411, 269–276.