# ANION BINDING: FROM SUPRAMOLECULES TO SENSORS

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## Natural anion binding receptors (proteins)

It is commonly understood that anions are highly important compounds in the living cycle of cell. Their influence on cellular biological processes is usually effected *via* macromolecular receptors, which are of protein nature. Anion-protein complex triggers a number of further biochemical transformations. In biological systems, the protein-anion interactions serve specific purposes, for example enzymatic transformations (where majority of enzymes bind anions as either substrates or cofactors), substance transportation, or signal transduction. They are usually noncovalent in nature. The high specificity of binding of anions to the respective ligands (proteins) is due to a recognition site in which anion is completely desolvated and bound exclusively *via* hydrogen bonds. Furthermore, a so-called macrodipole effect, caused by orientation of amino terminus of the protein backbones towards the negative guests, contributes to the stability of the complex. Then the oppositely charged functional groups of the protein and anions are paired, and lipophilic groups of the ligand display hydrophobic pockets formed by side chains of the hydrophobic amino acids[1]. The known anion binding proteins differ in structure, biochemical functions, and mechanisms of complex formation. The guanidinium group present in the side chain of arginine is ubiquitous in enzymes that bind anionic substrates and also involved in stabilization of protein tertiary structures via internal salt bridges with carboxylate functions[2]. This motif is realized in phosphatases and phosphorylases, which selectively bind phosphate, as well as in decarboxylases, dehydrogenases, isomerases, and in some proteases, that bind carboxylates. An analogous peculiarity is connected with the binding site structure of regulator proteins, the genes expression regulating factors; they bind with a high selectivity the certain sequences of nucleotides in DNA (Fig. 1).

Positively charged groups on the surface of nucleoproteins, the histones, are also capable to form strong (but not sequence specific) complex with such natural polyanion as DNA.

Some proteins contain metal cations (namely Zn(II), Fe(II), Mg(II), Ca(II)) in their anion binding sites. Such metal-containing proteins (metalloenzymes) form anion complexes as Lewis acid hosts, and exhibit selective binding with oxoanions (carboxypeptidases) or small inorganic anions, like $O_2^-$ (superoxide dismutase), $HCO_3^-$ (carboanhydrase), $Cl^-$ and $H^-$ (ionophores and cytochromes). Metal ions, as a $Zn^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Ca^{2+}$ play decisive role not only in binding anionic substrates, but also in the structure enforcement and all steric switching[2].

Anion binding by proteins can be achieved also by hydrogen-bonding and ion-dipole interactions, especially in the aqueous contents of the cell. A shining example can serve sulfate- and phosphate-binding proteins[3a]. The X-ray crystal structure showed that this anion was buried deep in the interior of the protein with the help of seven dedicated hydrogen bonds. There appears to be no functional group present in the binding cavity that could act as a hydrogen bond acceptor as required for binding of these anions. The transport of sulfate or phosphate anions through cell membranes is also regulated by neutral anion binding proteins[2].

## Artificial anion binding receptors

Recognition of anionic species has attracted the attention of many research groups. Research on the supramolecular chemistry of anions (selective recognition of guest molecules by synthetic receptors) has accelerated in recent years[4]. The design of host molecules for recognizing physiologically important anions, such as carboxylic acids or nucleotides, in

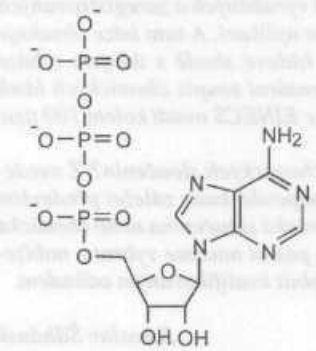
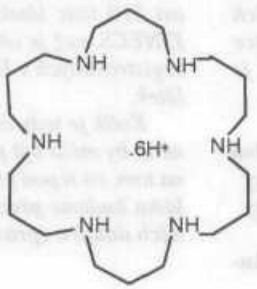
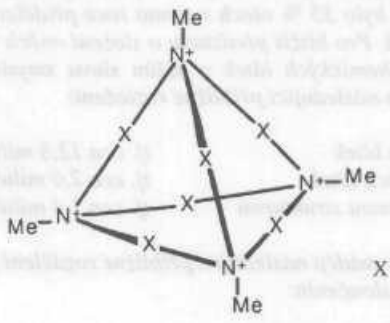



Fig. 1. **Adenosine triphosphate (ATP) is a biologically important anion**



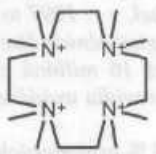




Fig. 2. Positively charged polyaza receptors **for halide** anions

aqueous solution is an important in view of the biological relevance of studies in this solvent[5].

All the known hosts have been categorized, according to their primary binding principles, into the positively charged and the electroneutral species. In this aspect, the extensive family of host molecules has been divided into three big groups. The first one comprises the positively charged anion hosts (Fig. 2).

The overwhelming majority of host compounds of this group are based on cationic nitrogen compounds. There are a few examples which suggest complexation by carbenium--based, iodinium, or sulfonium structures[2]. For this reason such well-known azonia compounds as different azamacrocycles (azacrown ethers and cryptands) are described. The binding of an anion by a crown ether can be improved by introduction of positive charges in the ligand. Positive charge can be introduced onto neutral crown ethers in two ways: either by protonation of nitrogen donor or complexation by metal ions[2]. Various derivatives of protonated azamacrocycles have shown host-guest binding effect with oxalate, sulfate, fumarate, citrate and some biologically important anions such as $AMP^{2-}$, $ADP^{3-}$, $ATP^{4-}$. Due to their ability to bind phosphate anions, the protonated polyamine azacrown ethers can be used to bind nucleotides ($ATP^{4-}$, $ADP^{3-}$ and $AMP^{2-}$). Some of these crown ethers (oxaazacrown ethers) were shown to cleave phosphoric anhydrides (ATP-ase activity)[2]. Several authors reported certain characteristic features of true enzymes which can be successfully modeled (catalytic turnover, saturation, and inhibition kinetics). With the aim of utilizing these macrocycles for ion sensing or membrane transport, they were attached to polystyrene resin or provided with $C_{16}$ hydrocarbon chains[2].

Such biologically important linear polyamines as spermine and spermidine also belong to this group of receptors. They are capable to bind phosphate and polyanions in water at neutral pH values. Their analogues, monocyclic hexamines, were designed to recognize dicarboxylic acid anions, and to show binding activity in aqueous media.

Further progress in the development of new azamacrocycles concerned the increase of their selectivity *via* covalent connection of two polyammonium macrocycles (assuming their cooperative action in anion binding), and rigidization of binding sites (bicyclic cryptates)[3a,c]. This route led to the formation of host molecules with augmented binding selectivity towards chloride, nitrate, phosphate and citrate anions in aqueous media. Another extension of utilization of water-soluble polyaza hosts series consisted in creating of their quaternary ammonium salts[2]. The anion binding using protonated polyaza hosts is severely hampered by the restriction to highly acidic pH regions, undermining thus any study involving the more basic anions. In addition, switch of the solvent may shift the $pK_a$ values and thus diminish the total charge by deprotonation, affecting thereby anion complexation[2]. Nitrogen quaternization might offer a remedy in this problem, and indeed the quaternary ammonium compounds were freely soluble in water without detectable aggregation and proved to be hosts for a broad variety of anions in water[2]. It is interesting, that some of these compounds were able to mimic in many respects the true enzymes (chemo- and substrate selectivity, saturation kinetics, inhibition, cooperativity, and turnover)[2]. The quaternary ammonium inclusion hosts are chemically rather stable compounds and opened the option to study reacting systems depending on host-guest binding without interference from pH effects.

Cationic cyclophanes[3a] are another example of hosts (Fig. 3).

The general binding principle for these anionic guests in water consist of a superposition of the hydrophobic effect and electrostatic attractions[2]. Such polyammonium macrocycles behave as efficient receptors for polycharged anions in aqueous solution[5]. In this case, preorganization of the macrocyclic host and the spatial charge matching have been recognized as primary conditions for strong host-guest interactions. In addition to charge-charge interactions, stacking, solvotropic forces and hydrogen bonds between anionic species and polyamine receptors can furnish additional contributions to complex stabilization[5]. Very strong and selective binding of anions containing a combination of hydrophobic and charged moieties as present in many biologically important guests, can be expected from the design of cyclophanes satisfying both binding requirements. This binding motif, when built into the cyclophane framework, provided a series of host compounds capable of binding nucleotides in water. Thus, for instance, the acridinium and phenanthridinium cyclophanes formed complexes in water with nucleotides and planar aromatic carboxylates[2].

Similar-type polycyclic cyclophanes were prepared from 1,3,5-trisubstituted benzenes with carbon chains containing secondary amines[3a]. These compounds can be solubilized in water by protonation, and formed complexes with numerous small inorganic anions such as nitrate, chloride, and sulfate. An interesting modification of cyclophanes on the basis of diazabicyclooctane and a further quaternization with hydrophobic moieties formed complexes with nucleotide triphosphates, and was capable of extracting ATP from a very dilute aqueous solution into the organic phase[2]. Their use in membrane transport experiments was not successful, as the detergenic properties of macrocycle led to disruption of liposome vesicles[2].

Side by side with azonia compounds, such positively charged anion hosts as oligopyrrole-derived receptors are widely known. Prominent examples among compounds of this family are polypyrrole complexes like metalloporphyrins and corrins. The anion-binding capacity of porphyrins depends on the presence of the metal ion. Small size of porphyrin cavity does not allow the use of N-H dipoles for anion stabilization. Expansion of the porphyrin cavity by the incorporation of more pyrrolic or other spacer moieties thus appeared as a rational remedy. A large number of ring-expanded porphyrins including the sapphyrins, were shown to possess anion-binding properties[6-12] (Fig. 4).

Diprotonated sapphyrin forms a very stable complex with fluoride, and with oxoanions such as phosphate it may form chelaton-type complex[6,7]. Open-chain and cyclic porphyrin

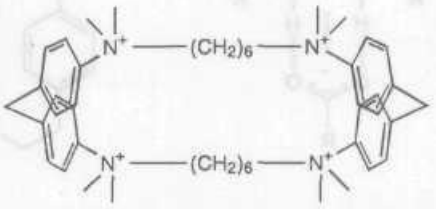



Fig. 3. A positively charged host for hydrophobic anion binding

dimers bearing various bisamide spacers (1,3-bisamidopropane, (1*S*,2*S*)-1,2-bisamidocyclohexane and (*S*)-2,2'-bisamido-1,1'-binaphthalene) were shown to display excellent chiral recognition properties for aspartate and glutamate anions[7]. Sapphyrin covalently linked to a solid matrix is useful in the separation of nucleotides, which allows for applying it in affinity chromatography. Even more promising application may derive from the ability of this compound to bind to single- or double-stranded DNA[13].

Various other modifications of porphyrins were also described. For instance, the porphyrins where two pyrrole rings were replaced by a rigid anthracene spacer, have a widened cavity, and are capable of more stronger complexation of halides[2]. In another case, using three porphyrin molecules as a building block, a giant cage molecule was obtained, which formed hexaprotonated cations with acids, and heteropolycluster anions such as $[PW_{12}O_{40}]^{3-}$, $[SiW_{12}O_{40}]^{4-}$ and $[Os_{10}C(CO)_{24}]^{2-}$. The all-*cis* atropoisomer of tetrakis(*o*-aminophenyl)porphyrin, as it was shown, gives complexes with chloride, bromide, nitrate, hydrogen sulfate and dihydrogen phosphate[14].

The increasing interest in the design of guanidinium-based receptors arose from investigation of catalytic mechanism of various enzymes (Fig. 5).

The guanidinium group is present in side chain of arginine in enzymes that bind anionic substrates and is also involved in the stabilization of protein tertiary structures via salt bridges with carboxylate function. Guanidine has an extremely high basicity, which guarantees protonation in a very wide pH range and a very effective solvation in water. Several bis- and polyguanidinium systems can form complexes with linear and cyclic phosphodiesters and mimic phosphodiesterase activity (which was shown in imidazole-catalyzed mRNA hydrolysis)[2]. Enhanced preorganization of host was employed in the design of a receptor for peptides. The 16-mer peptides with two aspartate groups located at different positions along the chain were tested for binding with the receptor, which was arranged as a rigid scaffold to orient two guanidinium groups[15].

In order to improve the binding characteristics, the guanidinium group can be embedded in a bicyclic framework. These bicyclic guanidinium groups formed ion pairs with a big number of anions[3]. They could be made also for the enantioselective recognition of amino acids, the catalysis, and specific transport of substrates across membranes. The same idea was used in the design of guanidinium nucleotide receptors. Attachment of uracil and naphthoyl subunits to the host molecule allows to get a receptor for adenine and the di- and oligonucleotides[2]. Another successful solution of tasks of anion recognition has been realized in the idea of linking two chiral guanidinium moieties with a flexible spacer. Such host molecules can extract dicarboxylates (succinate or fumarate), but not monocarboxylates, into the organic phase[2]. The host having bulky silyl ether groups is capable of extracting oxoanions from very dilute aqueous solutions. The highest preference was shown for sulfate, but $AMP^{2-}$, $ADP^{3-}$, and $ATP^{4-}$ were also extracted efficiently into the organic phase[16].

The introduction of a positive charge into organic frameworks as an alternative to protonation can be very efficiently accomplished by metal cation ligation. In this way, receptors with specifically designed coordination sites were constructed. When an organic chelating ligand binds to a metal cation, a mismatch of coordination sites may arise, which not necessarily spoils the complexation and may still give thermody-

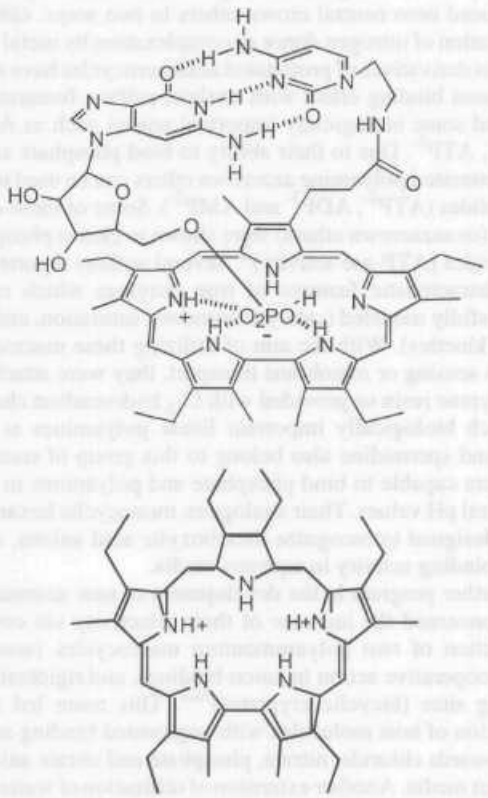


Fig. 4. Protonated sapphyrins acts as a receptor for anionic guests

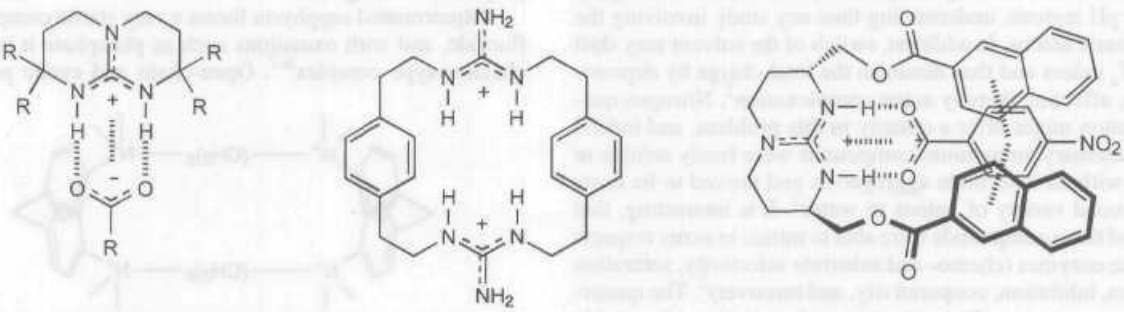


Fig. 5. A macrocyclic anion receptors containing guanidinium units

namically stable species[2]. For the formation of ion-pair the lower transition metal cations, notably Cu(I), Cu(II), Fe(II), Mn(II), Co(II), Ni(II), and Ru(II), have been preferred, although even the uranyl cation and the main group metals have been successfully employed, and there is no obvious limitation to the use of elements from the entire range of periodic table. Anion binding in these systems is thus widespread. A number of mono- and binuclear metallocomplexes on the basis of bipyridines (cyclophanes), calixarenes, and cobalto- or ferrocenes demonstrated binding affinity to a wide variety of anions (chloride, malonate, perchlorate, sulfate, azide) [3a-3c] (Fig. 6).

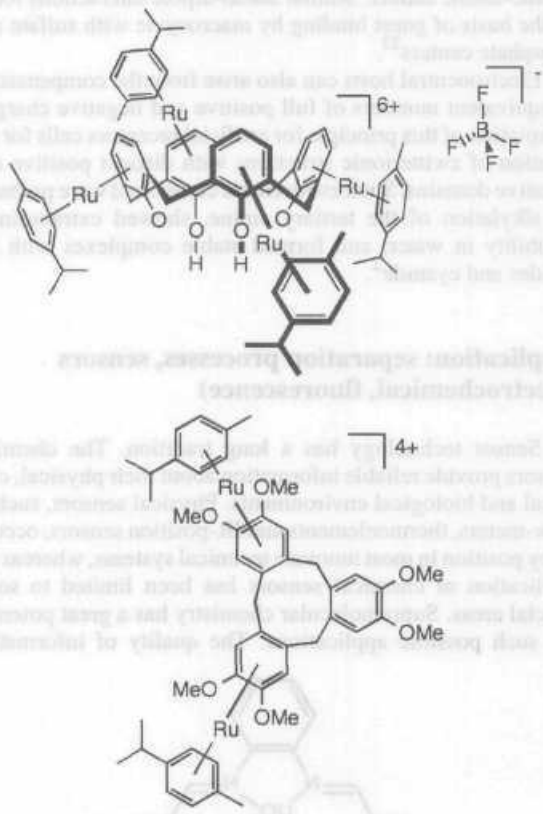



Fig. 6. Metal functionalizcd hosts

The calixarenes, cage-forming aromatic hosts, usually bind metal cations or neutral organic guests. Their guest preference can be inverted towards anions, providing that only strongly electron-attracting groups are attached to the aromatic rings (e.g. cations such as $Ru^{2+}$, and $Ir^{2+}$). Similar constructions can bind oxoanionic guests, like $CF_3SO_3^-$, $TcO_4^-$, and $ClO_4^-$ (Ref.[17]).

The classic representatives of host compounds intended for molecular recognition in water are cyclodextrins[4]. They provide toroidal cavities of variable size, and the guest molecules of correct complementary size can thus associate with this class of host compounds. Many inorganic anions, such as $ClO_4^-$, I', SCN", $CH_3COO^-$, $Cl^-$, $SO_4^{2-}$, form weak complexes with cyclodextrins[2]. Modification of these hosts with imidazole or pyridyl residues leads to intensification of their anion-binding characteristics. These modified cyclodextrins may serve as hosts for a variety of organic anions such as pyrocatecholates, sulfonates, carboxylates, or nucleotides. Some of these compounds are capable of mimicking the features of real hydrolytic enzymes[4].

To the second group, according to mentioned classification, belong the electroneutral hosts for anions (Fig. 7).

The neutral anion binding receptors can be divided into two classes: receptors that bind anions exclusively by hydrogen bonding or ion-dipole interactions, and receptors that coordinate anions as Lewis acidic centers of a neutral organometallic ligand [3a]. Among the representatives of this group, are Lewis acidic hosts connected by covalent bonds. At first, it is, e.g. boron Lewis acids, combined with naphthalene or crown ether. Such a construction of a host molecule provides selective binding of hydride and halide ions[2]. Borane groups, replaced in macrocycles on trimethylsilyl moieties exhibited

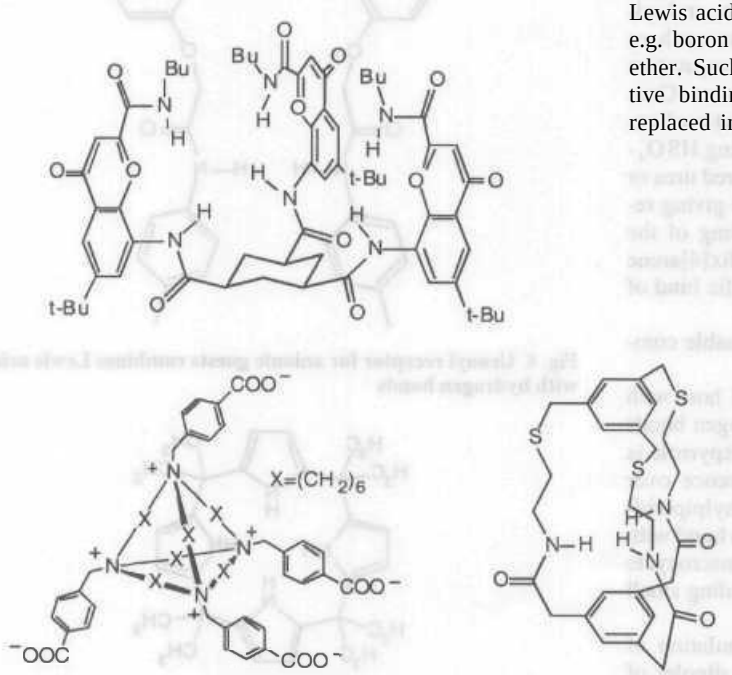



Fig. 7. Neutral receptors species for anion binding

selective interaction with fluoride ion[2]. Tin(IV)macrocycles have been characterized as capable of binding chloride anion[2]. Organic tin(IV) compounds are known to possess an affinity to phosphate[18]. A favorable candidate for use as an architectural element can be seen in mercury. It forms unusually stable carbon bonds extending from the metal[2]. With such macrocyclic hosts, the halides complexes are easily formed[2]. The Lewis acidic hosts based on metal cation coordination also belong to this group. These are systems of neutral overall charge. Tetranuclear Cu(I) complex, created on the basis of rigid resorcinarene phosphonite ring, encapsulates chloride guest anion[2]. A very similar complex was formed with Ag(I)--macrocycle and iodide. Large transition metal cations allow for anchoring of the guests into an organic polychelate while leaving still one coordination site open for additional binding of a Lewis base[2]. Complex $Zn^{2+}$-*N*-methylmesoporphyrin binds *N*-acyl-α-amino acid anions as guest species with strong enantioselectivity[2]. Uranylsalenes (Fig. 8) displayed selectivity toward $H_2PO_4^-$ (Ref.[3a,19]). These host compounds were successfully tested as carriers in experiments concerning transport of phosphate anions across a supported liquid membrane[2]. Thus the utility and advantages of this design of electroneutral anion receptors was demonstrated.

*Anion hosts operating by ion-dipole binding* represents the third group of artificial receptors family. Ion-dipole interaction has the same dependence on the dielectric environment as the interaction partners bearing full charges, but is considerably weaker on the absolute scale and decreases with the distance more steeply[2]. Energy of the interaction of an ion and a dipole depends on their mutual orientation. Hydrogen bonds are the most prominent representatives of dipolar elements and play a very important role in biopolymers. They serve to "glue" host and guest together, especially in less polar solvents. As very helpful compounds in this case can be viewed the urea-base and flexible tentacle polyamine hosts, which complexate with perchlorate, phosphate, carboxylate, and sulfonate structures and with isosteric oxo-structures such as lactone and nitro ones[2]. Well-known and enjoying reputation as metal cation complexing agents are the calixarenes. Connecting of the sulfonamide function to the upper rim of calix[4]arene produced a host capable of distinguishing HSO4--ion from the chloride or nitrate[2], and joining tethered urea or thiourea moieties to calix[4]arene or calix[6]arene giving receptors showed a strong and regioselective binding of the 1,3,5-benzenetricarboxylate[2]. Modification of calix[4]arene upper rim by urea moieties lead to effect of specific bind of the halide anions[3b].

Calixpyrrole is a well-known and easily obtainable compound (Fig. 9).

It has been characterized as an electroneutral host with cone structure enabling to form cooperative hydrogen bonds of all pyrrole groups with the anionic guests. Calixpyrrole is capable of binding fluoride with a strong preference over chloride or $H_2PO_4^-$ (Ref.[20]). The neutral pentamethylpiperidine receptor[2] also formed four amide-like hydrogen bond with cyclohexanedione enolate anion. A steroid-based macrocycle was designed on this principle. It is capable of binding small halide anions[21].

Hydrogen bonding is sensitive to the accumulation of negative charge density and able to employ bond dipoles of heavier, non-hydrogen elements[2]. In order to make use of this interaction principle, a macrocyclic tertiary amine containing four borane-amine donor bonds was prepared[22]. A large number of inorganic anions were successfully complexed by this borane-amine adduct. Similar anion-dipole interactions formed the basis of guest binding by macrocycle with sulfate and phosphate centers[23].

Electroneutral hosts can also arise from the compensation of equivalent numbers of full positive and negative charges. Adaptation of this principle for artificial receptors calls for the creation of zwitterionic structures with distinct positive and negative domains. Such zwitterionic compound were prepared by alkylation of the tertiary amine, showed extraordinary solubility in water, and formed stable complexes with the halides and cyanide[2].

## Application: separation processes, sensors (electrochemical, fluorescence)

Sensor technology has a long tradition. The chemical sensors provide reliable information about their physical, chemical and biological environments. Physical sensors, such as flow-meters, thermoelements and IR-position sensors, occupy a key position in most innovate technical systems, whereas the application of chemical sensors has been limited to some special areas. Supramolecular chemistry has a great potential for such possible applications. The quality of information

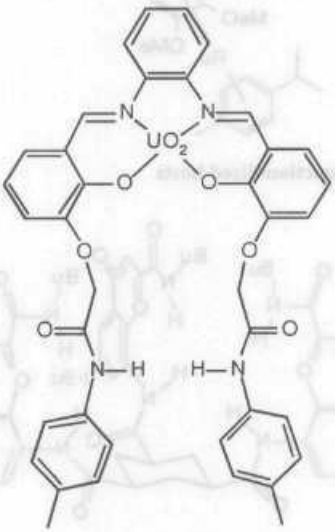



Fig. 8. Uranyl receptor for anionic guests combines Lewis acidity with hydrogen bonds

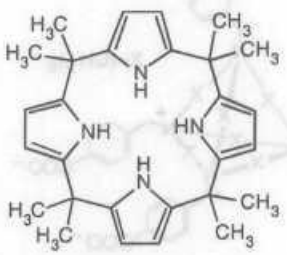



Fig. 9. *meso*-Octametylcalix[4]pyrrole

provided by chemical sensors is fundamentally different from that supplied by traditional analytical methods. In some cases, this has been the source of unexpected uncertainties in comparing methods. Chemical sensors have allowed the identification and quantification of specific species and have had a great impact on the study of biological systems. Anion receptors are now applied as the selector element in separation membranes and in ion-selective electrodes. As one of such uses the application of macrocyclic oligopyrrole compounds are their use in molecular recognition for HPLC separation of biologically important anionic substrates can be mentioned[24]. This application usually requires the immobilization of receptors on a polymeric matrix or silica gel. The covalent attachment of these receptors to a solid phase provides valuable tool for understanding receptor-substrate interactions and gives possibility to determine the thermodynamic and kinetic factors of particular binding. The modification of silica-gel by attachment of receptor subunits can lead to the generation of supports that show differing affinities toward various anionic species[40]. Here, such suitable receptors as porphyrins, metalloporphyrins, sapphyrins, calix[4]pyrroles and binaphthyl derivatives were employed[4,20,40]. These macrocycles (with carboxyl functions on the periphery) were bonded to aminopropyl-silica gel *{via* amide bonds) and then silylated. For comparison of their separation properties, bonded phases with various amounts of macrocyclic receptors were prepared[2]. Separation modes are based on hydrogen bond formation, axial ligand and coulombic interactions and π–π hydrophobic interactions.

Host-guest molecular recognition on membrane surfaces is a fundamental chemical process that controls many important biological reactions, such as antigen-antibody reactions, enzymatic reactions, carrier- or channel-mediated transport, and receptor reactions involving signal transduction. From the viewpoint of analytical chemistry, it gives important implications for the sensing of target chemical substances[25,26]. An important method for *in situ* analysis of these substances is a measurement of the guest-induced membrane potential change. The ion-selective electrodes (ISE) are now being intensi vely exploited. The electrode system that is most widely used is a liquid membrane type, in which a lipophilic organic liquid membrane containing lipophilic host molecules is generally supported by a poly vinyl chloride (PVC) polymer matrix. For practical applications different types of liquid membranes, namely bulk, supported, emulsion and polymer composite can be used[26]. Calix[4]pyrroles and metalloporphyrins with appended cytosine unit were proposed as receptor of ion-selective electrodes for nucleotide recognition[27]. The molecular recognition of nucleotides is usually based on complementary base pairing, directed multiple hydrogen bonding, specific stacking, and generalized electrostatic interactions which act either individually or cooperatively. The described receptors were designed as nucleotide receptors since the recognition process involves multiple binding interactions. It was found that membranes, containing substituted calix[4]pyrroles gave near-Nernstian responses in the presence of both 5'-GMP and 5'-CMP and demonstrated the following selectivities: 5'-AMP<5'--UMP<5'-TMP<5'-GMP<5'-CMP and 5'-AMP<5'-GMP< 5'-UMP<5'-TMP<5'-CMP, respectively. On the other hand, the PVC-membranes based on cytosine-substituted metalloporphyrins (Co(II) and Zn(II)) displayed high selectivity only to 5'-CMP and similar selectivity to others nucleoside monophosphates. In order to understand complex mechanism of binding between metalloporphyrins and nucleotide the unsubstituted Co(II)-tetraphenylporphyrin have been studied. Experimental data suggest that potentiometric response of unsubstituted metalloporphyrin is controlled by both nucleobases coordination and phosphate chelation.

The most interesting aspect of the potentiometric selectivity appeared to be in the combination of Co(II)-porphyrin and lipophilic additive (such as tridodecylmethylammonium chloride (TDMACl)). These results indicate, that cytosine-substituted metalloporphyrin, and in particular the Co(II)-containing one, may be potentially useful for determination of complementary nucleotide in the presence of 10 mol% TDMACl.

The open-chain and cyclic sapphyrin dimers bearing various bisamide spacers are also interesting as receptors for chiral dicarboxylate anions[28]. The chiral diamines should provide an additional source of both hydrogen-bonding donor (i.e., NH) and acceptor (i.e., C = O) functionality that could complement the "main" sapphyrin-to-carboxylate binding motif. In view of this, (*S*)-2,2'-diamino-1,1'-binaphthalene and (1*S*,2*S*)-1,2--diaminocyclohexane as stereogenic linkers in these systems were chosen. Chiral recognition of unprotected and protected (N-carbobenzyloxy, N-Cbz) aspartate and glutamate anions by these compounds was examined by incorporating sapphyrin-based receptors as active components into plasticized PVC-membranes. Obtained data indicate, that enantioselectivity of the diaminocyclohexane-derived dimers toward glutamate is higher than that of the binaphthalene-containing receptor, and that the enantioselectivity of both open-chain and cyclic dimers to N-Cbz-aspartate is controlled by plasticizer polarity.

Among ion-selective sensors capable of serving as carriers in the transfer of oxoanions to nonpolar organic phase, rubyrin[29], lipophilic bis-guanidines[30], and macrotricyclic quaternary ammonium ions $RMQA^{4+}$ weredescribed[31]. The hosts proposed for the imitation of membrane transport exactly and selective transfer of halide and phosphate guests from the aqueous environment to a PVC or phospholipide membrane phase.

Other electrochemical sensors, the aromatic boronic acids, interacted with $Cl^-$ and F" in solid matrices and organic solution[32]. For electrochemical detection of fluoride ions in aqueous media ferrocenboronic acid[32] was prepared. Chloride ions have virtually no effect on the electrode potential, and neither have thiocyanate, phosphate, sulfate and others. The complexation is caused by the strong Lewis acid - Lewis base interaction. Hydroxide ions react, in analogy to fluoride ion, with the boronic acid; but this occurs only in strongly alkaline solutions and another redox reaction occurs in parallel. The ferroceneboronic acid has similar excellent selectivity as anion selective electrodes based on $LaF_3$ but the principle is different[32]. Ferroceneboronic acid may find also industrial application as an inexpensive and highly selective sensor for fluoride anions. On the basis of others halide anions sensors are cobaltocenium polyamide macrocyclic receptors, bis-cobaltocenium porphyrin- and calix[4]arene derivatives, or calix[4]arene with Ru(II)-bipyridyl moiety, attached *via* amide bonds[3b-c,33]. They exhibit selectivity trend in the order $Cl^- > Br^- >> NO_3^-$. A new class of anion selective receptors based on the neutral uranylsalophene building block as Lewis acidic binding site was described[34,35]. Incorporation of these novel ion receptors in PVC membranes on chemically modified field

effect transistore (CHEMFETs) allow determination of phosphate and fluoride anions in aqueous solutions, even in presence of anion excess of very lipophilic ions such as nitrate and perchlorate. CHEMFETs are silicon-based microsensors that can transduce the membrane potential of an ion selective membrane deposited on top of the semiconductor into an electronic signal. The new series of uranilsalenes contained of different anion binding groups, like amides, urea moieties, and Lewis acidic metal centers which combined both cation and anion binding properties also was proposed[36]. These ditopic receptors were tested for complexation studies of inorganic anions and can be applied as useful sensor element in potentiometric measurements.

Molecular recognition of such a natural poly anion as DNA was recently shown on examples such as porphyrins and sapphyrins[4]. The positively charged porphyrins and protonated expanded sapphyrins are capable of interacting with nucleic acids in several interesting ways. The main DNA binding modes of porphyrins are electrostatic interaction between positively-charged substituents of porphyrins with the polyanionic DNA sugar-phosphate backbone, the hydrophobic interaction and intercalation. In the case of sapphyrins three modes of the interaction with DNAs have been found: phosphate anion chelation by the protonated core of sapphyrin, hydrophobic interaction, and at the low phosphate ester to sapphyrin ratios the formation of highly ordered aggregation of sapphyrin on the surface of certain dsDNAs (Ref. [36]). Further progress in this area could provide novel polypyrrole-conjugates with oligonucleotides [37]. The macrocyclic units attached to an oligonucleotide can serve a dual role: it can act as a sequence-specific modification agent and also increase the affinity of a macrocyclic-bearing oligonucleotide to a complementary sequence.

Compounds incorporating a binding site and a fluorophore, and which dispose of a mechanism for communication between the host and guest, are called fluorescent chemosensors[38]. These sensing molecules contain the bipyridyl, naphthalene, anthracene or acridine cores, attached *via* polyamine moieties. Such receptors have the potential to give a highly sensitive light-emitting response on complexation of a large range of organic anions (including such polyanions as DNA or heparin)[38]. Binding selectivities of fluorogenic chemosensors, combined with their photophysical properties, offer attractive features for its use as fluorescent sensors for the detection of anions and polyanions, especially in the case of biological targets involving nucleic acids [39].

## Conclusion

The anion receptors are very diverse as to their chemical nature, ranging from macromolecular proteins to relatively low molecular synthetic macrocycles. The structures of anion binding sites in the molecules, as well as binding modes, are also highly different. A lot of anion hosts are able to bind biologically important anions in anion enzyme-mimic manner. Their features are very useful in a variety of applications. Among the anion carriers have found a particularly wide field of application as components in anion-selective membrane electrodes, e.g. in clinical chemistry (solvent polymeric membrane electrodes)[41,42] or electrophysiology (microelectrodes)[43,44], as detectors in ion chromatography[45,46], in highly selective transport processes through artificial and biological membranes. As a result of the introduction of synthetic ionophores as anion-selective components in membrane based sensor systems of analytically relevant selectivity, the direct measurement of $Cl^-$, $CO_3^{2-}$, $NO_2^-$, $NO_3^-$, $ClO_4^-$, $NCS^-$, $HCO_3^-$, $BF_4^-$ has become available [47]. The direct extra- and intra-cellular measurement of $Cl^-$, $NO_3^-$, and $CO_3^{2-}$ have also been rendered possible with neutral ionophore-based microelectrodes[43,44]. The study of anion supramolecules and their derivatives has been fruitful in a number of various new macrocycles produced. The study of the interaction of these new hosts with a variety of guests has opened many ways of research and has led to a number of promising applications.

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**V. Král, O. Rusin, T. Shishkanova, R. Volf, P. Matějka, and K. Volka** *(Department of Analytical Chemistry, Institute of Chemical Technology, Prague):* **Anion Binding: From Supramolecules to Sensors**

The article deals with the recent synthesis of a host of reagents for anion and polyanion binding and describes the whole area of use ranging from analytical chemistry and environmental science to medicinal applications.