The Cation–π Interaction

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Contents

I. Introduction

Noncovalent interactions play a dominant role in many forefront areas of modern chemistry, from materials design to molecular biology. A detailed understanding of the physical origin and scope of such interactions has become a major goal of physical organic chemistry. Compared to the more conventional interactions such as hydrogen bonds, ion pairs (salt bridges), and the hydrophobic interaction, the cation–π interaction[1] has been relatively underappreciated. It is not a new effect—experimental support for a prominent interaction in the gas phase appeared more than 15 years ago,[2] and the potential for such an interaction has always been evident from an electrostatic analysis of benzene.

However, it was not until cation–π interactions were convincingly demonstrated and quantitated in a condensed phase, and especially in aqueous media, that the potential for this broadly applicable and important noncovalent binding force was recognized. The first indications came from two directions: an analysis of protein crystal structures;[3] and studies involving artificial, cyclophane receptors in aqueous media.[4–6] These early observations have led to a detailed investigation of all manifestations of the cation–π interaction, from simple, gas-phase ion–molecule complexes to large, multisubunit protein systems. It is now clear that cation–π interactions are prominent in a wide range of systems and should be considered as an important and general noncovalent binding force.[1,7]

The present review will provide a detailed overview of the cation–π interaction. We will first describe the fundamental nature of the interaction, emphasizing gas-phase measurements and high level theoretical studies. These provide valuable insights into the nature of the cation–π interaction and suggest a novel electrostatic model that can serve as a useful guide for predictions about new systems. We then describe studies using synthetic receptors, mostly cyclophane structures in aqueous media. These systems provide the best quantitative data on the cation–π interaction in a realistic medium. Most importantly, these studies unambiguously established that a hydrophobic binding site comprised of aromatic rings can compete with full aqueous solvation in the binding of highly solvated cations. Given these results, it was then reasonable to expect that Nature would use cation–π interactions in appropriate situations, and it is now abundantly clear that this is so. We will catalogue the many biological systems in which cation–π interactions are, or could well be, important in molecular recognition. The recognition of the generality of the cation–π interaction has led to a better understanding of several novel binding sites, and to new suggestions about catalytic mechanisms.

Note that in discussing the cation–π interaction, we are not considering complexes of transition metals such as Ag^+, Fe^+, etc. with benzene and related π systems.[8–10] It is clear that the bonding in such systems is quite different from the effect we are discussing here because of interactions with d orbitals on the metal. In fact, it seems unreasonable to consider complexes such as $Ag^+\cdots C_6H_6$ as being dominated by noncovalent interactions. Also, the reader should keep in mind that while the emphasis

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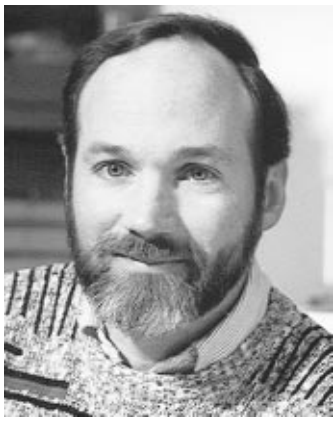


Dennis A. Dougherty was born and raised in Pennsylvania, and received a BS/MS in 1974 from Bucknell University. He received a Ph.D. from Princeton University in 1978, working with Kurt Mislow, and this was followed by a year of postdoctoral work with Jerome Berson at Yale University. In 1979 he joined the faculty at the California Institute of Technology, where he is now Professor of Chemistry.

will be on the binding of cations to aromatic systems, the cation−π interaction is not restricted to them—it is not a "cation-aromatic" interaction. Ethylene, acetylene, and other simple π systems are fully anticipated to and are documented to be involved in cation−π interactions.

II. Fundamental Studies

A. Gas-Phase Measurements

The fundamental nature of the cation−π interaction is best revealed by gas-phase studies of ion−molecule complexes. Long before cation−π interactions were recognized in cyclophane hosts or protein crystal structures, high-pressure mass spectrometry and ion cyclotron resonance studies established that cations bind strongly to simple aromatic systems. Table 1 lists measurements for all such complexes for which one can be fairly certain the cation is bound to the π face of the aromatic. For example, a pyridine···Na^+ complex has been characterized,[11] but is not included in Table 1, because it is highly likely that the Na^+ binds to the N lone pair, not to the π system.

The magnitudes of these interactions are quite substantial—competitive with what one might expect from even the strongest of noncovalent binding forces. Still, without some kind of external reference, it is difficult to know to what extent cation−π interactions might be important in a condensed phase. Fortunately, the pioneering work of Kebarle[2] provided the perfect reference compound—water. All would agree that the interaction of an ion such as K^+ with water should be strong in the gas phase, and it is. The K^+···water interaction energy is 18 kcal/mol. But, the K^+···benzene interaction energy is 19 kcal/mol! In fact, throughout a series of ion···molecule complexes with K^+ binding from one to four molecules of benzene or water, the benzene complexes are stronger.[2] More recently, more sophisticated techniques have allowed the preparation and characterization of clusters that contain both benzene and water molecules around a single ion. Invariably in such clusters, the more weakly bound molecules are the *waters*—they are the first to be removed by collisional activation.[12]

Subsequently, important studies by Meot-Ner established that more complicated cations can also show strong affinities to simple aromatics.[13,14] NH_4^+ binds to benzene with an interaction energy comparable to that of K^+. These two ions—NH_4^+ and K^+—are similar in other ways, including aqueous solvation energies and permeabilities through selective ion channels. Alkylation of the ammonium ion diminishes the cation−π interaction, but even NMe_4^+ shows a substantial binding energy of 9 kcal/mol. As with K^+, the NMe_4^+···water interaction is quite comparable to the NMe_4^+···benzene interaction (Table 1). Along with K^+, benzene complexes of Li^+ [11] and Na^+ [8] have been observed, producing an informative trend that will be discussed below. It has even been suggested that in protonated benzene ($C_6H_7^+$), the most stable structure has the proton over the center of the ring, not attached to a carbon.[15]

B. Computational Studies

High-level theoretical studies have provided an excellent complement to the experimental, gas-phase work. To date, no experimental information on the geometries of these ion−molecule complexes has been obtained. Hopefully, in the near future sophisticated techniques of the kind that have provided geometries of the benzene···water[16] and benzene···ammonia[17] complexes will be applied to cation−π complexes. Fortunately, ion−molecule complexes of the sort in Table 1 are amenable to high level theoretical studies. The wealth of thermodynamic data available provides a demanding test of any theoretical model. If a theoretical model successfully reproduces a series of binding energies, one can expect that the calculated geometries will also be in good agreement with the experimental structures. All levels of theory agree that the preferred geometry for a simple cation interacting with benzene places the cation over the center of the ring, along the 6-fold axis (Figure 1).

There has been some discussion of what level of theory is adequate for evaluating cation−π interactions. While semiempirical methods such as AM1 seem to reproduce trends across a series of related compounds, they may not be generally reliable for quantitative studies.[18] For complexes of simple ions

Table 1. Gas-Phase Ion Molecule Binding Energies[a]

A. Experimental Measurements

ion	molecule	binding energy[b]	ref	ion	molecule	binding energy[b]	ref
Li^+	C_6H_6	38.3[c]	11	NH_4^+	C_6H_5F	(14.4)[i]	13
Na^+	C_6H_6	28.0	8	NH_4^+	1,4-$C_6H_4F_2$	(13.0)[i]	13
K^+	C_6H_6	19.2	2	NH_4^+	C_2H_4	10.0	13
$K^+ \cdot C_6H_6$	C_6H_6	18.8	2	$CH_3NH_3^+$	C_6H_6	18.8	13
$K^+ \cdot (C_6H_6)_2$	C_6H_6	14.5	2	$CH_3NH_3^+$	cyclohexene	11.6	13
$K^+ \cdot (C_6H_6)_3$	C_6H_6	12.6	2	$CH_3NH_3^+$	pyrrole	(18.6)[i]	13
K^+	H_2O	17.9	2	$(CH_3)_3NH^+$	C_6H_6	15.9	13
Al^+	C_6H_6	35.2	26	NMe_4^+	C_6H_6	9.4	14
NH_4^+	C_6H_6	19.3	13	NMe_4^+	$C_6H_5CH_3$	9.5	14
NH_4^+	1,3,5-$C_6H_3(CH_3)_3$	21.8	13	$C_2H_5OH_2^+$	C_6H_6	21.0	13

B. Computational Results

ion	molecule	binding energy[m]	ref	ion	molecule	binding energy[m]	ref
Li^+	C_6H_6	43.8[k]	22	NH_4^+	C_6H_6	19.1[k]	22
Li	C_6H_6	39.5[l]	19	NH_4^+	C_6H_6	17.9[g]	23, 24
Li^+	C_2H_4	24.3[k]	22	NH_4^+	C_6H_6	16.3[d]	13
Na^+	C_6H_6	29.5[k]	22	NH_4^+	C_6H_6	17.2[d]	18
Na^+	C_6H_6	24.4[l]	19			22.2[e]	
Na^+	C_6H_6	27.1[j]	20	NH_4^+	C_6H_6	19.0[f]	25
Na^+	C_6H_5F	20.0[j]	20	NH_4^+	$C_6H_5CH_3$	20.5[f]	25
Na^+	C_6H_5OH	26.9[j]	20	NH_4^+	C_6H_5F	11.4[d]	13
Na^+	$C_6H_5NH_2$	31.8[j]	20	NH_4^+	C_6H_5OH	20.5[f]	25
Na^+	$C_6H_5BH_2$	24.4[j]	20	NH_4^+	indole	25.9[f]	25
Na^+	C_6H_5Cl	21.5[j]	20	NH_4^+	C_2H_4	10.9[d]	13
Na^+	C_6H_5CN	15.7[j]	20	H_3O^+	C_6H_6	19.4	37
Na^+	1,4-$C_6H_4F_2$	16.8[j]	20	$CH_3NH_3^+$	C_6H_6	18.3[f]	25
Na^+	1,3,5-$C_6H_3F_3$	12.4[j]	20	$CH_3NH_3^+$	C_6H_6	5.7[d]	18
Na^+	pyridine	20.0[j]	20			8.3[d]	
Na^+	pyrrole	29.6[j]	21	$CH_3NH_3^+$	$C_6H_5CH_3$	19.7[f]	25
Na^+	imidazole	21.0[j]	21	$CH_3NH_3^+$	C_6H_5OH	18.2[f]	25
Na^+	indole	32.6[j]	21	$CH_3NH_3^+$	indole	25.7[f]	25
Na^+	furan	21.0[j]	21	NMe_4^+	C_6H_6	15.4[k]	22
Na^+	thiophene	22.8[j]	33	NMe_4^+	C_6H_6	10.2[g]	23, 24
Na^+	naphthalene	28.7[j]	20	NMe_4^+	C_6H_6	10.8[f]	25
Na^+	azulene	34.1[j]	21	NMe_4^+	C_6H_6	8.4[d]	18
Na^+	$C_6H_5OH\cdots$formamide[k]	36.1[j]	21			11.3[e]	
Na^+	cyclohexane	8.4[j]	21	NMe_4^+	$C_6H_5CH_3$	11.9[f]	25
K^+	C_6H_6	15.0[k]	22	NMe_4^+	C_6H_5OH	10.7[d]	18
K^+	C_6H_6	19.2[l]	19			16.3[e]	
Rb^+	C_6H_6	15.8[l]	19	NMe_4^+	indole	15.8[f]	25
Al^+	C_6H_6	39.0[h]	26	NMe_4^+	indole	11.7[d]	18
						16.3[e]	

[a] All energies in kcal/mol. Only cation–π complexes considered—other structures are also possible for some complexes. [b] ΔH, unless otherwise noted. [c] Calculated from $\Delta G°$ values, assuming $\Delta S° = 23$ eu. [d] HF-3-21G. [e] Corrected for BSSE and dispersion energy. [f] 6-31G*//3-21G-MP2. [g] 6-311 + G*-MP2 plus corrections. [h] 6-31G(d,p)-MP2. [i] Geometry unknown—may not be cation–π complex. [j] 6-31G**//6-31G**. [k] MP2/6-31G*//MP2/6-31G*. [l] 6-31G** for C and H; STO-3G* for ion. [m] ΔE values.

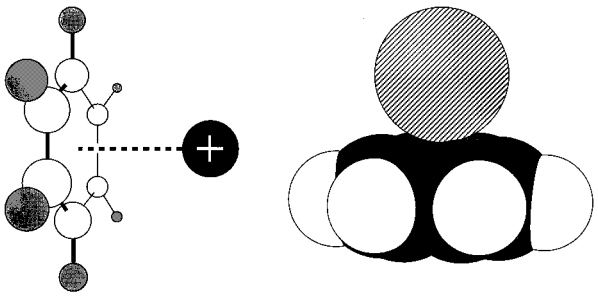


Figure 1. Schematic of the cation–π interaction: (left) the basic interaction showing a generic cation positioned over benzene along the 6-fold axis and (right) a space-filling model of the $K^+\cdots$benzene complex at its optimized geometry,[19] showing the essentially van der Waals contact between the two.

such as Li^+, Na^+, and K^+, it appears that single-determinant *ab initio* theory with an extended basis set such as 6-31G** is adequate.[19–22] On the other hand, with more complex "organic" cations such as NMe_4^+, correlation effects of the type included in an MP2 calculation may be important if quantitatively accurate results are desired.[23–26]

Table 1 lists a number of calculated cation–π binding energies, spanning a range of theoretical methodologies. Again, only π complexes are given. For a number of systems, structures with the ion bound to a heteroatom lone pair are also viable, and perhaps even stronger than the cation–π complex.[21] For cation–π complexes, the agreement between theory and experiment is generally excellent where comparisons are available. This engenders considerable confidence in the higher levels of theory, allowing one to use the computational studies to evaluate many aspects of the cation–π interaction.

A number of Na^+ complexes of simple aromatics have been evaluated with high level *ab initio* calculations (Table 1), allowing a systematic evaluation of

"substituent effects" in the cation−π interaction.[20,21] One might have anticipated that classical aromatic substituent effects derived from studies of electrophilic aromatic substitution would be relevant to the cation−π interaction, since both involve a cation interacting with an aromatic. This, however, is decidedly not the case. Most telling are phenol, which is no better than benzene in a cation−π interaction, but is substantially more reactive in electrophilic aromatic substitution; and furan, which is a weaker cation−π binder than benzene, but is *much* more reactive in electrophilic aromatic substitution. Given these findings, it is not surprising that no Hammett parameters of the sort that emphasize resonance effects can fit the trend in cation−π interactions. There is a fairly good fit using σ_{meta}—a parameter that emphasizes inductive rather than resonance effects.[20] These trends and others will be discussed in the following section, where we attempt to develop a physical model for the cation−π interaction.

Recently, several computational studies of cation binding to much more complex aromatic systems have appeared. For the bowl-shaped semibuckminsterfullerene (triindenotriphenylene), binding of ions such as Li^+, Na^+, and K^+ to both the concave and convex faces should be considered. Interestingly, ab initio and semiempirical methods sometimes disagree as to which face is preferred.[27,28] Binding of Li^+, Na^+, and K^+ to the beautiful spheriphane first prepared by Vöglte[29] has been studied by high level ab initio methods, and both endohedral and exohedral structures are seen.[30]

Given the relevance of cation−π interactions to a number of biological structures (see below), there is special interest in the simple aromatics that model amino acid side chains. These are benzene (Phe), phenol (Tyr), indole (Trp), and imidazole (His). Of these, indole is clearly the strongest cation−π binder (Table 1), suggesting that Trp may be especially important in cation−π binding. It is interesting that electrostatic maps[1,21] and quantitative calculations[20] indicate that the benzene ring of indole is the preferred cation−π binding site over the 5-membered pyrrole-type ring. While the model calculations predict that Phe and Tyr should be very similar in cation−π binding ability, further studies showed that if the OH of Tyr is hydrogen bonded (modeled by phenol···formamide, Table 1), the cation−π binding strength is substantially increased.[21] Also from a biological perspective, it would be very valuable if modeling packages aimed at proteins and nucleic acids would treat such interactions reliably. However, Jorgensen found that OPLS parameters that are quite successful in modeling benzene···water and tetramethylammonium (TMA)···water, could not properly model benzene···TMA,[31] a result presaged by our work on K^+···benzene complexes.[19] Consistent with this, Kollman has shown that "non-additive effects" are essential in such modeling.[22] However, non-additive terms are computationally costly, and it has been found that alternatively, addition of an appropriate "10−12" function into the AMBER field can lead to useful modeling of cation−π interactions.[32]

C. A Physical Model for the Cation–π Interaction

In our own work, we first encountered cation−π interactions in the binding of organic cations such as tetraalkylammoniums and alkylpyridiniums to cyclophane receptors (see below). Our bias then was that "donor−acceptor" or "charge-transfer" interactions would dominate the cation−π interaction. However, subsequent study, especially of gas-phase complexes, established that electrostatic interactions play a prominent, and sometimes dominant role in prototypical cation−π interactions. There has been some confusion concerning the "electrostatic model" of the cation−π interaction, and so we shall present a detailed description of it here.

1. The Electrostatic Model

A clear indication that electrostatics play an important role in the cation−π interaction comes from a comparison of simple alkali metals binding to benzene (Table 1). the trend is $Li^+ > Na^+ > K^+ > Rb^+$. This is a classical electrostatic sequence—exactly what would be seen if the benzene were replaced by Cl^- or if one was comparing aqueous solvation energies. If polarizability, dispersion forces, or charge-transfer effects were dominant, one might have expected the larger Rb^+ ion to be the strongest binder.

Electrostatic reasoning can also explain variations due to changes in the aromatic ring. Our first use of electrostatics in this regard came in our efforts to explain the surprisingly poor cation−π binding ability of furan and thiophene, both in the gas phase and in cyclophane hosts.[33] These "electron rich" heterocycles are, in fact, quite poor cation−π binders (Table 1). We found that, qualitatively, the benzene−furan−thiophene series could be rationalized by inspection of the electrostatic potential surfaces of the aromatics. The more negative the maximum in electrostatic potential over the center of the aromatic, the stronger the cation−π interaction.

A subsequent, systematic study clearly established a prominent role for electrostatics in the cation−π interaction. In particular, across a series of 11 aromatics, 100% of the variation in cation−π binding energy for Na^+ was due to variation in the electrostatic component of the binding.[20]

It is important to understand the nature of these findings. It is *not* true that 100% of the cation−π binding energy is electrostatic. In fact, the fraction of the total binding energy that is electrostatic varies considerably, depending on the aromatic. In simple, prototype systems it is a substantial component. Kollman showed that for the Li^+···ethylene complex ~60% of the binding is due to electrostatics.[22] We found a similar fraction for the Na^+···benzene complex.[20] However, for other systems such as Na^+··· (1,3,5-trifluorobenzene) there is *no* electrostatic component to the cation−π interaction. It is the *variation* in ion-binding energies that is faithfully mirrored by the electrostatic term. If one simply wants to predict the *trend* in a cation−π interaction across a series of similar aromatics, all with the same cation, one need consider only electrostatics.

It is for this reason that visual inspection of electrostatic potential surfaces provides a reliable

qualitative guide to cation–π interactions.[21] In a number of instances we have shown that these colorful maps are quite useful in discussions of cation–π interactions. There are a number of potential pitfalls in this inherently qualitative approach, but it can be useful.

The "nonelectrostatic" component of the cation–π interaction, sometimes the major component, reflects a combination of "effects" mostly related to the polarizability of the aromatic. Probably the most important of these for simple systems is the interaction of the ion with the induced dipole in the aromatic.[34] Donor–acceptor and charge-transfer terms along with dispersion forces may also be important. Note that with a large organic ion such as NMe_4^+, polarizability terms will be more important than with Na^+.[23,24]

While these "nonelectrostatic" terms are always important, what is remarkable is that across a series of aromatics they make a *constant* contribution to the total binding energy.[20] While polarizability of the aromatic is important, it is decidedly *not* the defining feature of the cation–π interaction. Benzene is not an especially polarizable molecule, and in fact, it is more polarizable *in plane* than perpendicular to the ring.[35] Cyclohexane is well known to be substantially more polarizable than benzene,[36] but it is certainly not a better cation binder (Table 1). Also, explicit studies of the role of an induced dipole in cation binding showed that cyclohexane is better than benzene in this regard—it is just that the induced dipole is not enough to make a strong cation binding site.[37]

The electrostatic model rationalizes most of the trends of Table 1. Concerning the aromatic component, we have shown that electrostatic potential surfaces of the aromatic rings are excellent indicators of the trends in cation–π interactions.[21] Variations among the ions are also consistent, in that the cation–π interaction decreases as the ionic radius increases, as expected for an electrostatic model. In this light, the substantial drop on going from NH_4^+ to NMe_4^+ can be viewed as resulting from the large increase in ionic radius. There is no indication of any special N–H (or CH)$\cdots\pi$ interaction—no need to invoke a hydrogen bond to benzene. Whether the ion is Li^+, NH_4^+, or NMe_4^+, the same fundamental interactions are involved.

2. The Quadrupole Moment

In an effort to clarify the nature of the electrostatic component of the cation–π interaction, we, following the insightful analyses of Reisse[38] and Williams,[39] emphasized the role of the large, permanent quadrupole moment of benzene. This has led to some confusion, which we will attempt to clarify here.

For most organic chemists, to describe a molecule as "polar" is equivalent to saying it has a permanent dipole moment. A molecule with a dipole, such as water, experiences a favorable interaction with an ion, if the ion is positioned near the appropriate end of the dipole. If one does not allow any electronic reorganization of the molecule or the ion, this interaction is purely electrostatic.

Benzene, of course, has no dipole moment, but it does have a substantial, permanent quadrupole moment.[40] Recall that a quadrupole can be thought of as two dipoles aligned in such a way so that there is no net dipole. Topologically, quadrupoles are equivalent to d orbitals (as dipoles are to p orbitals, etc.), and the quadrupole in benzene in particular is topologically equivalent to a d_{z^2} orbital. Thus, there is a permanent, nonspherical charge distribution in benzene, with regions of relative negative and positive charges. Plots of the electrostatic potential surface provide a useful way to visualize the quadrupole.[1,21,33]

Just as an ion can be attracted to the appropriate end of a dipole, so can an ion experience a favorable interaction with appropriate regions of a quadrupole. This is an electrostatic interaction—it requires no adjustment of the electronic distribution around the ion or the molecule. Importantly, there is no *a priori* reason to expect that such interactions will be inherently weaker when the molecule contributes a quadrupole rather than a dipole. In fact, model calculations by Reisse[38] established that a point charge experiences a comparable stabilization whether interacting at long range with a point dipole significantly larger than that of water or a point quadrupole comparable to that of benzene. Similarly, Williams has shown that the quadrupole provides an important force for controlling solid-state architecture.[39,41]

That said, *the cation–π interaction cannot be quantitatively modeled as just an ion–quadrupole interaction*. That is, the physics of an ion–quadrupole interaction are not appropriate for a cation–π interaction. The reason for this is the short interaction distance typically involved in a cation–π interaction (Figure 1). The representation of the electronic distribution of a molecule as a multipole expansion, which is then used to evaluate electrostatic interactions,[42] is valid only at large interaction distances. Hence, the model calculations noted above[38] involved a dimensionless (point) quadrupole interacting with a point charge 5 Å away. In contrast, a real cation–π interaction of the sort shown in Table 1 involves a cation that is typically at van der Waals contact with the aromatic. For example, in the $Na^+\cdots$benzene complex, r (the distance from the center of the cation to the center of the benzene ring) is ca. 2.4 Å. In such a case, the dimensions of the quadrupole (e.g., the distance between the two centers of negative charge) are comparable to r. As such, any quantitative argument based on a multipole expansion is completely inappropriate. Consistent with this conclusion, an ion–quadrupole interaction is expected to show a $1/r^3$ distance dependence[42,43] for the stabilization energy. Instead, we find that the distance dependence of a prototype cation–π interaction is $1/r^n$ with $n < 2$, clearly not an ion–quadrupole interaction.[1] At the close distances of a cation–π interaction, one must evaluate the interaction of the ion with the full electrostatic potential surface of the molecule in order to quantify the electrostatic contribution to binding.

The usefulness of the quadrupole moment is that it provides an easy way to visualize the charge distribution of aromatics and leads naturally to the expectation of significant electrostatic interactions. It also correctly predicts the preferred geometries of

cation−π complexes and other "polar−π" interactions.[43−47]

While the presence of a finite quadrupole moment in benzene is mandated by symmetry, the origin of the large magnitude of the moment may not be obvious. A useful model is simply to conclude that sp^2 C is significantly more electronegative than H. In benzene, this produces six local bond dipoles which, in combination under the symmetry of the system, give rise to a molecular quadrupole. This view allows a novel interpretation of some of the trends in the data of Table 1.[48] For example, one could argue that the weak cation binding of heterocycles such as pyridine, furan, and thiophene is due to the loss of a C−H bond dipole. In contrast, introducing a strong N−H bond dipole as in pyrrole enhances the cation−π interaction. This view leads naturally to the expectation that ethylene and acetylene should be good cation binders, as they are.

III. Artificial Receptors

Supramolecular recognition in solution has been an extensively studied field.[49−51] Molecular interactions between small molecules and artificial receptors have been characterized using NMR spectroscopy, UV/vis/fluorescence spectroscopy, circular dichroism techniques, and X-ray crystallography. These methodologies have long been established and will not be discussed. Of relevance here, these methods have provided a large number of quantitatively reliable determinations of binding affinities. Still, the use of such approaches to establish the importance of the cation−π interaction is not entirely straightforward. This is because many different forces contribute to molecular binding, and it is often difficult to delineate the contribution from a specific interaction. Some other important molecular interactions are the hydrophobic interaction (in water), classical electrostatic terms such as ion pair and ion−dipole interactions, donor−acceptor interactions, dispersion and van der Waals forces, etc. In any binding event, some or all of these forces contribute to the recognition. In order to firmly establish a cation−π interaction, great care must be taken to separate the importance and contribution of each of these effects.

For this reason, the most compelling evidence for cation−π interactions comes from systematic studies of a series of related structures. A single observation of a strong binding interaction between a host and a guest can be subject to many interpretations. However, when a large series of measurements consistently points to an important role for cation−π interactions, one can be more confident of their importance.

A. Studies in Aqueous Media

A large number of studies of synthetic receptors (Figure 2) in aqueous media have documented the importance of the cation−π interaction. In water, a cation will be very well solvated. The binding cavities of the synthetic receptors must compete with aqueous solvation if a cation is to bind tightly. This "desolvation penalty" is quite substantial (see below), but through a combination of cation−π and other interactions, strong binding of organic cations in aqueous media can be seen.

The importance of studies in aqueous media is that they can have significant implications for biological recognition. If a synthetic receptor can pull an organic cation out of water and into a nominally hydrophobic binding site, then perhaps Nature can adopt a similar strategy. As we will document below, this is certainly the case.

Studies in aqueous media present special challenges also. One is the prominent role of the hydrophobic interaction. This large effect can often dominate binding studies in aqueous media, and establishing a significant contribution from other forces in the face of a strong hydrophobic effect can be difficult. Also, in order to make synthetic receptors water soluble, one must often append polar groups that are typically charged. The possibility of conventional electrostatic interactions between cationic guests and these polar groups can complicate analysis of binding studies.

The most extensive studies of cation−π interactions in aqueous media have been based on a series of cyclophane hosts (e.g., **1**−**4**) developed by the Dougherty group.[4−6,33,52−55] These structures are soluble and monomeric at pH 7−9 by virtue of the remotely positioned, anionic carboxylates. In 1986, it was noticed that the quaternary ammonium ion ("quat") adamantyltrimethylammonium **20** (Figure 3) bound tightly to host **1**.[4] This result was surprising at the time, as very few aliphatic guests had been bound by cyclophane hosts. Soon thereafter, a systematic study of quinolinium-type structures established the importance of cation−π interactions in such binding.[5] (At the time, we referred to this as an "ion−dipole" interaction, an unfortunate choice of terms that we later abandoned in favor of "cation−π".) An important observation in this early study was that replacement of two of the aromatic "walls" of **1** by cyclohexanes (to produce **2**) seriously impaired the cation binding ability of the host. This established that it was the aromatic nature of the host—not the remote carboxylates—that was responsible for cation binding. This result also presaged the preference of the electrostatic model discussed above over the alternative model that emphasizes the importance of an induced dipole (i.e., polarizability). As noted above, cyclohexane is more polarizable than benzene.[35,36] If the induced dipole effect dominated the cation−π interaction, **2** should have been a better binder of cations than **1**.

These initial observations launched an extensive study of such interactions. Studies of over a dozen cyclophane hosts have produced over 150 binding constants for a wide array of ammonium, iminium, guanidinium, and sulfonium guests. Highlights include:

• The comparison of the "isosteric" guests **21** and **22**.[5] Host **1** binds the cationic **21** more tightly than **22** by 2.5 kcal/mol, even though the cationic guest is better solvated than the neutral by 46.5 kcal/mol.[55]

• In guest **23** it is the polar $-NMe_3^+$ group rather than the hydrophobic $-CMe_3$ group that inserts into the hydrophobic cavity of **1**.[6]

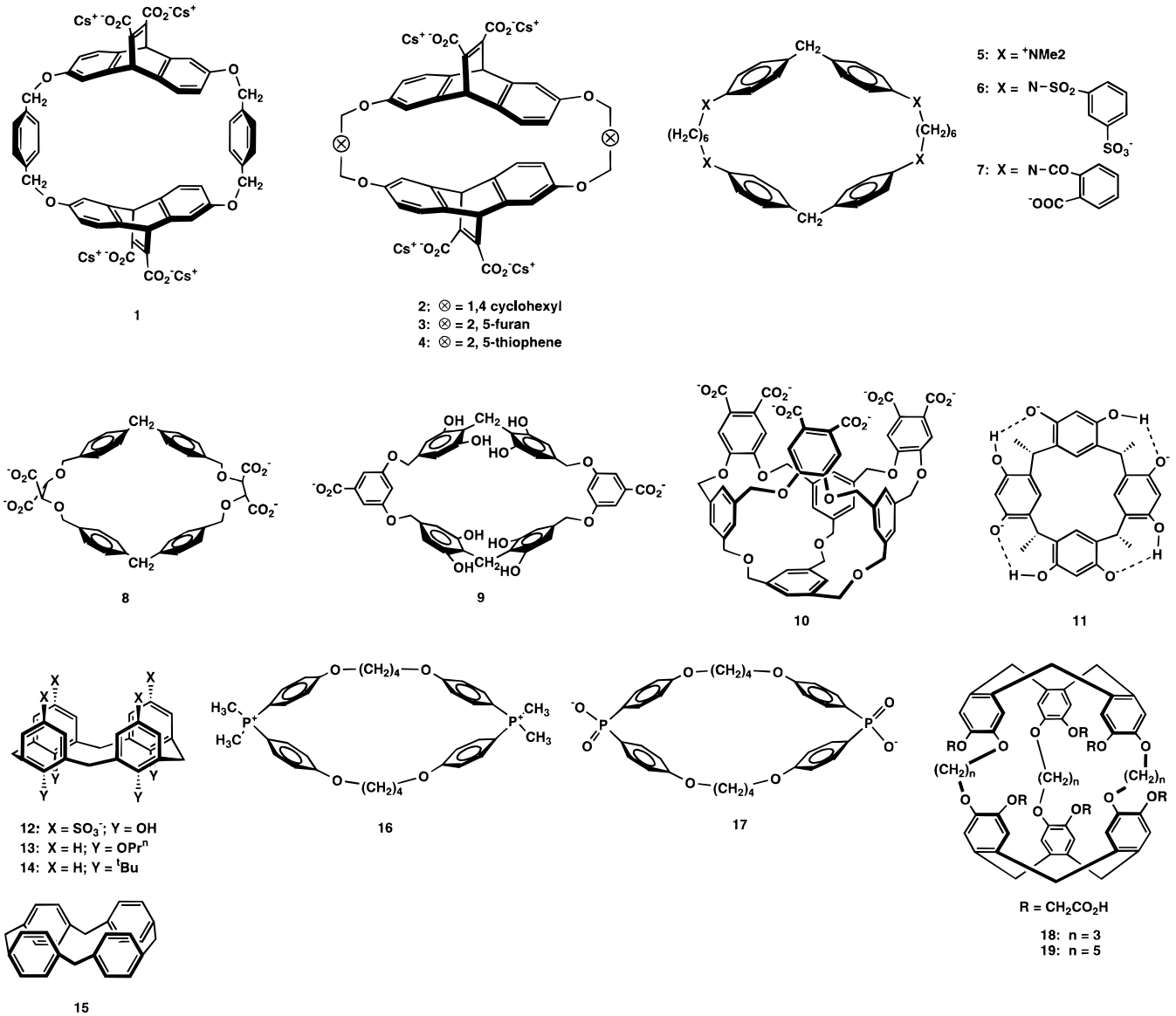


Figure 2. Synthetic receptors used to characterize the cation–π interaction in aqueous media.

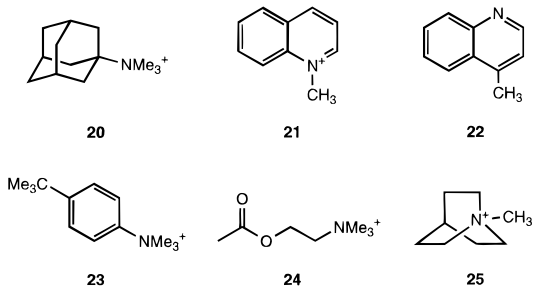


Figure 3. Representative guests for the receptors of Figures 2 and 4.

• Replacing two benzene rings of **1** by "electron rich" furan (**3**) or thiophene (**4**) rings led to a *decrease* in cation binding.[33] This was at first considered surprising, but is now recognized to be completely consistent with the electrostatic model described above.

• The biologically relevant quat acetylcholine (**24**, ACh) binds to **1** with an affinity comparable to that of natural ACh receptors.[55] This has significant biological implications, discussed more fully below.

Important contributions to our understanding of cation–π interactions have also come from studies by the Schneider group.[34,56–62] They have convincingly demonstrated strong arene–ammonium interactions in a series of studies on azacyclophane host **5** (Figure 2). This positively charged, lipophilic host binds arenes and nucleosides in aqueous solution. Schneider also found that the complexation energy between host **5** and naphthalene is significantly greater than the complexation with naphthalene's saturated analogs, tetrahydronaphthalene and decalin.[61] In each case the binding energy decreases by 1–1.3 kcal/mol for the removal of an aromatic unit. Since these substrates have similar hydrophobic surface areas, the preferential binding of the aromatic substrates must derive from the favorable interactions with the π electrons.

The positively charged alkylammonium ions on the host proved to be important in the binding interaction. Anionic hosts **6** and **7** were also synthesized and their complexation energies to different aromatic substrates determined.[59,61] The anionic charges were incorporated via benzyl groups to remove them from the immediate proximity of the binding cavity. For neutral aromatic substrates such as naphthalene, the host with the positively charged cavity (**5**) was a

significantly better receptor than the negatively charged host (**6**) with a neutral cavity. However, the two hosts showed similar affinities for aliphatic guests, for which hydrophobic interactions would be the primary driving force.

Schneider's observation of cation−π interactions in these systems led to a series of experiments intended to quantify this interaction.[56,60] Binding energies between a selection of aromatic and aliphatic compounds with positively or negatively charged functional groups were determined. When cation−π interactions were possible, substrates with aromatic rings showed additional binding energies that were proportional to the number of phenyl rings involved. From these studies, Schneider determined that the cation−π interaction contributes ∼0.5 kcal/mol of binding energy per phenyl group.

Lehn's group has, of course, performed extensive studies on host−guest recognition, including studies relevant to the cation−π interaction.[63−67] The hosts are primarily cyclophanes with anionic solubilizing functional groups (Figure 2). The speleand-type host (**8**), for example, forms a 1:1 complex with a variety of alkylated ammonium ions including acetylcholine at neutral pH. Other polyphenolic (**9**) or catechol-type (**10**) cyclophane hosts also bind quaternary ammonium and some iminium ions well. In these hosts, as in some of those described by Schneider (**11**),[68,69] Shinkai (**12**) (see below), and others,[70−72] the anionic groups used to solubilize the host can make very close contact with cationic centers on guests. As such, it is difficult to quantify the relative importance of cation−π vs more conventional ion pair interactions. Still, the Lehn hosts provide some of the strongest binding constants for acetylcholine and related structures.

The strategy of using charged hosts to bind aromatic substrates was later adopted by Schwabacher.[73] The cyclophane hosts **16** and **17** (Figure 2) were designed to be very similar in structure but to carry opposite charges. Unlike Schneider's or Dougherty's anionic hosts, which have charges far removed from the cavity that served mainly to solubilize the host, the negative charges on host **16** are adjacent to the binding cavity and can actively participate in binding. The charges were positioned to be along the edge of an aromatic guest, where the positive region of the quadrupole moment was directed. The authors found that in 60:40 D_2O/CD_3OD, the anionic host binds aromatic guests such as naphthalene more than 5-fold stronger than the cationic host.

Like others who studied the cation−π interaction, Shinkai first observed this effect while studying hydrophobic interactions.[74−80] At low pH's the *p*-sulfonatocalix[4]arene **12** (Figure 2) binds the phenyl group of the trimethylanilinium guest, but at pD 7.3 there is a significant amount of inclusion of the trimethyl head group. At higher pH the phenols are ionized, and the calixarene π system becomes much more electron rich. This apparently enhances the cation−π interaction enough to overcome the hydrophobic interaction that dominates complexation at the lower pH. Complementary NMR studies of neutral calixarenes in organic solvents[74] show that the positively charged end of trimethyloctylammonium is the portion bound inside the cavity. Very recently, a crystal structure of acetylcholine bound to a neutral calixarene prepared by Aoki clearly shows cation−π interactions between the quat of the guest and aromatics of the host.[81]

Organic guest binding is observed in the gas phase as well. Positive secondary ion mass spectrometry (SIMS) clearly detects the **13**/guest$^+$ complex ion peak.[76,77,80] Similar gas-phase studies established calixarene binding of simple metals ions, including an apparent selectivity for K^+ in some systems. However, in these gas-phase studies it was difficult to distinguish cation−π binding from complexation by oxygens of the calixarene, and crystal structures of the calixarene−metal complexes showed the cation and the ether oxygens to be in close proximity.[75,82]

To differentiate the cation−oxygen and the cation−π interaction, Shinkai synthesized two more calix[4]arenes (**14** and **15**) (Figure 2) that contained only hydrocarbon substituents. These hosts were mixed with alkali metal and silver cations and analyzed by SIMS and positive electrospray ionization mass spectrometry (ESI-MS).[77] Signals for the **15**/Rb^+ and **15**/Cs^+ complexes were very intense compared to the signals for **15**/Li^+, **15**/Na^+, and **15**/K^+ complexes, consistent with the fact that the cavity size of cyclophane **15** matches the ionic radius of Cs^+ most closely.

Several recent computational studies are especially relevant to molecular recognition studies of the type described here. Modern simulation studies of NH_4^+···toluene[32] and of tetramethylammonium···benzene[31] both showed that even in such simple complexes the cation−π interaction can overcome the substantial aqueous solvation energy of the cation to produce a stable complex. These results further support the notion that prototypical cation−π interactions are viable in aqueous solution.

B. Studies in Organic Solvents

The previous section illustrates a serious constraint to working in aqueous media—typically one must functionalize the cyclophane for solubility, thereby introducing other potential molecular recognition elements. Many researchers have taken to examining the cation−π interaction in organic solvents to avoid the complication of distinguishing the contribution from Coulombic interactions with the solubilizing groups. The organic solvents also eliminate the large hydrophobic driving force that dominates affinity in aqueous media.

The Dougherty group conducted a series of binding studies with the neutral tetramethyl ester of host **1** in chloroform (Figure 4).[53] This neutral host, **26**, binds positively charged substrates such as **20** and **21** with appreciable affinity. The neutral substrates, quinoline and isoquinoline, are not bound at all, suggesting that the positive charge is critical to binding. Similarly, the nonfunctionalized cyclophane host **27** synthesized by Mandolini's group (Figure 4) was found to bind quaternary iminium and large aliphatic ammonium ions well.[83] The best substrate is the *N*-methylquinuclidinium ion **25**. Simple CPK modeling suggests that the globular shape of this ion best fills the cavity of host **27**. In addition, the

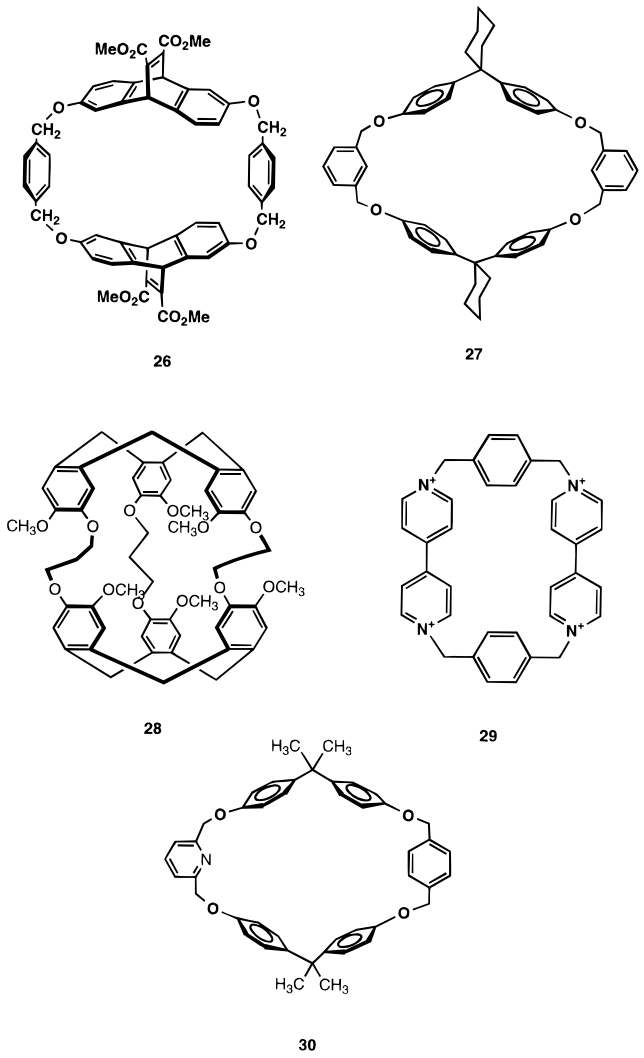


Figure 4. Synthetic receptors used to characterize the cation–π interaction in organic media.

hexaester analog of Lehn's catechol host binds **20** in chloroform.[65]

A spectacular example of cation–π interactions in organic media is provided by Collet's studies of the cryptophane host **28** (Figure 4).[84–86] In $(CDCl_2)_2$, the hexaester host binds NMe_4^+ with remarkably high affinity, $-\Delta G° = 7.4$ kcal/mol. The cryptophane hosts were also studied in an aqueous environment where the methoxy substituents were converted to carboxylates. The anionic host **19** (Figure 2) complexes choline, acetylcholine, and other quaternary ammonium ions with high affinity. Collet found that the smaller cryptophane **18** has much lower affinity for the ammonium substrates. They suggest that the cation–π interaction in this case may not be strong enough to compensate for the entropy decrease in forming the ordered complex. Thus a larger cavity gives stronger binding, leading to the novel conclusion that loose association is actually preferred over the traditional concept of tight lock and key fitting.[86]

Entropy loss proves to be an important issue in carlixarene-based hosts, which can adopt a variety of conformations. As such, crown ether linkers have been used to confer more rigidity. In $CDCl_3$, calixacrown hosts have been shown to bind a variety of quaternary ammonium ions.[87–89] NMR shifts and in some cases NOE experiments demonstrated that the methyl and the methylene protons near the charged nitrogen were bound inside the aromatic cavity. Binding studies with calixarene hosts of varying sizes also seem to suggest that a more preorganized cavity improves ammonium binding.[90] Additionally, a calixarene host was designed to mimic the phosphocholine binding site of the antibody McPC603 (see below) by incorporating a guanidinium ion near an aromatic-rich cavity.[91] These hosts have shown high affinity toward acetylcholine and a variety of phosphocholine derivatives in chloroform. Additionally, a crystal structure of a calixa-crown binding K^+ suggests a cation–π interaction between the K^+ and one of the aromatics of the calixarene.[92]

Cation–π complexes have also been observed while studying the intermediates in the reaction between nitrosonium cations and arenes.[93,94] Nitrosonium salts in TFA at room temperature oxidize naphthalene to form a radical cation monomer which then polymerizes. Extensive NMR studies suggest that a π complex between the arene and the nitrosonium ion is formed prior to the electron transfer. The same nitrosonium π complex was also suspected to be the intermediate in the nitrosation reactions of anisole and thioanisole. In fact, the NMR studies indicate that while the nitrosonium cation interacts with the sulfur atom on thioanisole, it binds to anisole over the aromatic ring.

Cation–π interactions may also be involved in the paraquat-based self-assembling structures that have been so spectacularly developed by Stoddart.[95–97] In 1988 it was observed that paraquat based cyclophanes, such as bisbipyridiniumbixylylcyclophane **29** (Figure 4), are able to complex with neutral guests like 1,4-dimethyoxybenzene. The complex had little contribution from conventional electrostatic interactions, and the crystal structure suggested only weak edge-to-face interactions between the paraphenylene units and the aromatic protons of the guest. The primary binding contribution is attributed to a dispersive interaction between the electron-rich aromatic system of the guest and the electron poor host. A unique feature of these systems is that the paraquat is so electron deficient, that in both organic and aqueous media, complex formation is accompanied by the development of visible charge-transfer bands, perhaps signaling a qualitative change in the nature of the binding interaction.

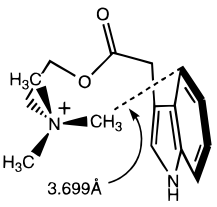
In a clever study by Parker and Rosser, an uncharged cyclophane host, **30** (Figure 4), was synthesized and incorporated into a PVC–*o*-nitrophenyloctyl ether-based membrane.[98] A Nernstian response to acetylcholine transport across the membrane was observed. Ion transport was not observed for NMe_4^+, NEt_4^+, or in the absence of the cyclophane host. Although binding studies of host **30** by NMR spectroscopy were unsuccessful, the demonstration that this host can mediate selective acetylcholine transport across a membrane is remarkable.

Gokel and co-workers have explored the contribution of aromatic residues in synthetic ion channels.[99,100] They synthesized a series of three-ring azacrown-based artificial ion channels and studied their ion transport rate by monitoring the sodium

cation flux through phospholipid bilayer vesicle membranes. They found that the channel with benzyl side arms produced 40% greater ion flux than the channel with the lipophilic dodecyl sidearms. The authors suggest that the benzyl groups probably stabilize the extended channel conformation by interacting with the polar head groups of the membrane.

C. Solid-State Examples

Several examples of cation−π interactions have been documented in studies of small molecule crystal structures. To investigate the contribution of the trimethylammonium substituent in the recognition between acetylcholine and its esterase, Aoki's group synthesized two isosteric model compounds. Indole-3-acetic acid choline ester (**31**), was designed to test the interaction of the ammonium ion and the aromatic indole ring.[101] 3,3-Dimethylbutyl indole-3-acetate (**32**), is the control compound missing the positive charge. Molecule **31** crystallizes in a folded conformation. The indole ring and the ammonium



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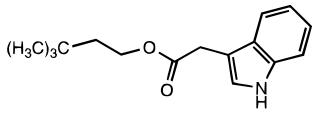

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ion are making close contact at 3.699 Å between C(13) and C(5). Furthermore, the neighboring indole ring is packed next to the ammonium tail with 3.429 Å between C(14) and N(1). In contrast **32** crystallizes in the fully extended form where the head and the tail groups are far apart. There is no intermolecular interaction between the *tert*-butyl group and the indole ring in the crystal lattice. The comparison suggests that the electronic difference, namely the positive charge, contributes significantly to the difference in the crystal structures of the two molecules.

Verdonk et al. later searched for similar interactions in the Cambridge small molecule structural database, and found that the radial distribution of the phenyl−onium distance peaks sharply at 4.6 Å and then at 6 Å.[102] Within these two groups of interaction distances, there are two different orientations: at 4.6 Å, the amine lies along the C_6 axis of the phenyl ring; and at 6.0 Å the amine interacts off the C_6 axis.

In several other small molecule crystal structures, stabilizing interactions between ions such as Li^+, K^+, and Cs^+ with simple aromatic rings have been observed.[103−106] In addition, a number of structures of cationic forms of heavier elements such as P, Ga, Sn, and Ge show strong interactions between the cation and aromatic rings.[107−111] Although analyzing the factors that contribute to crystal packing is always complex, cation−π interactions are likely contributors in these systems.

IV. Cation–π Interactions in Biological Structures

A. Side-Chain Interactions in Proteins—the Amino–Aromatic Interaction

In pioneering work, Burley and Petsko[3] observed a preference for NH groups on amino acid side chains to be near aromatic side chains (Figure 5). A statistical analysis of the distance between the nitrogens in the side chains of Arg, Lys, Asn, Gln, and His and the aromatic residues, Phe, Tyr, and Trp, was performed on high resolution structures of 33 diverse proteins. Roughly 50% of the aromatic residues in these proteins were in close contact (less than 6 Å) with amino groups, and more than 25% of the Lys, Asn, Gln, and His residues were in van der Waals contact with aromatics. More impressively, 50% of the Arg residues were in contact with an average of two aromatic side chains. The authors dubbed this an "amino−aromatic" interaction, and favored a predominantly electrostatic model.[43]

The amino−aromatic interaction was presaged by important observations of Levitt and Perutz.[112−114] In a study of hemoglobin−drug interactions, they observed a "hydrogen bonding" type of interaction between aromatic π electrons and the N−H of an amide group. Empirical potential functions suggested that the amide−benzene hydrogen bond is worth approximately 3 kcal/mol, with the nitrogen−benzene distance between 2.9 and 3.6 Å. They proposed that aromatic rings can serve as general hydrogen-bond acceptors.

Subsequent studies have refined and expanded upon the earlier analysis of Burley and Petsko. Thornton's group analyzed 52 high-resolution protein structures and found a high distribution of side chain nitrogen atoms from Asn, Gln, His, Lys, and Arg around 3.6 to 3.8 Å from aromatic rings.[115] Although there is a preference for this interaction, the authors did not find the hydrogen-bonding amino−aromatic interaction to be especially prominent. For example, for amino acids containing sp^2-hybridized nitrogen (all the above except Lys), the stacked geometry is favored over the perpendicular, hydrogen-bonding geometry (Figure 5) by a 2.5:1 ratio.[116,117]

Extensive, general statistical analyses of side-chain interactions in 186 nonhomologous, well-resolved protein structures by Karlin[118,119] confirmed that the guanidinium−aromatic interaction is mostly stacking. Additionally, there appears to be a special preference for a cation−π interaction involving Arg and Trp. While in general there is a preference for interplanar contacts with the 5-atom ring of tryptophan, arginine prefers the 6-atom ring. This is consistent with the above theoretical studies showing that the 6-membered ring of the indole is the better cation−π binder of the two. Karlin also found that Tyr and Trp are over-represented as nearest neighbors of both Lys and Arg. All of the aromatic residues emphasize Arg among their over-represented neighbors. Lysine is also a common neighbor of aromatics, although the lysine−tryptophan interaction often involves a CH_2 group of the Lys, rather than the NH_3^+.

We would advocate breaking the amino−aromatic side chain−side chain interaction into two classes.

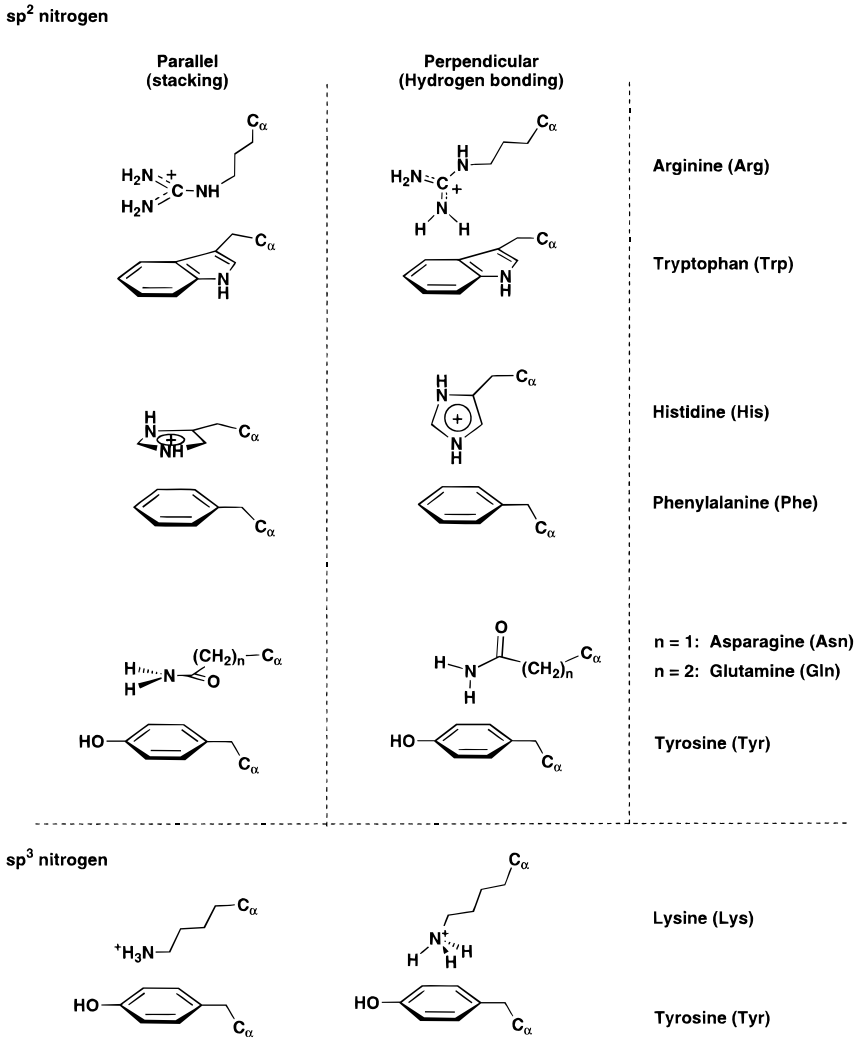


Figure 5. Side-chain structures for amino acids involved in amino–aromatic interactions. Prototypical, limiting geometries are shown. Structures are illustrative only, and are not intended to indicate preferences for particular geometries or combinations of amino acids.

When a cationic side chain is involved (Arg, Lys, or protonated His), this is an example of a cation–π interaction, which is dominated by the kinds of electrostatic interactions discussed above. Such interactions can be quite strong (see Table 1). Note also that the CH_2 directly adjacent to the positively charged groups of Lys and Arg carries a substantial positive charge (like a methyl of a NMe_3^+ group) and so contact between this CH_2 and the face of an aromatic will be a cation–π interaction. On the other hand, for neutral nitrogen-containing side chains (Asn and Gln) the interaction with aromatics will be *much* weaker. For example, the relevant gas-phase reference for this kind of interaction is the $NH_3\cdots C_6H_6$ complex, with a binding energy of 1.4[17] vs the 19 kcal/mol binding energy of $NH_4^+\cdots C_6H_6$.[13]

Of particular interest is the interaction of the cationic Arg residue with aromatic side chains. Two limiting geometries are possible—a perpendicular arrangement in which the NH's of the Arg point into the face of the aromatic, and a parallel or stacked arrangement of the planar guanidinium of Arg and the aromatic (Figure 5). The stacked arrangement seems especially interesting because of a possible hydrophobic contribution. Intriguing calculations by Wipff indicated that two guanidinium ions in aqueous media tend to associate in a face-to-face geometry.[120] This is presumably due, in part, to the fact that the face of a guanidinium is a delocalized π system, a hydrophobic structure. Although gas-phase calculations indicate that the perpendicular, hydrogen-bonding arrangement of a guanidinium/benzene pair is more energetically favorable than the stacking interaction,[31,117] this preference is completely reversed in aqueous simulations.[31] A large part of the reversal is due to the better hydrogen bonding to water by the guanidinium in the stacked geometry. Consistent with this, analysis of 62 high resolution protein structures showed that arginine residues often hydrogen bond to other carbonyl groups while stacking above the face of aromatic rings.[121] Flocco and Mowbray saw a similar bias for stacking when they analyzed arginine–aromatic interactions.[122]

Since the publication of the various statistical analyses, a number of more recent crystal structures have provided numerous examples of the arginine–aromatic stacking interaction, and a few Arg–aromatic hydrogen bonding interactions.[123–131] Highlights include: observation of both geometries in lactoferrin;[132] an Arg/Trp interaction in the tyrosine kinase domain of the human insulin receptor;[133] and an Arg stacking on an adenine base in the complex

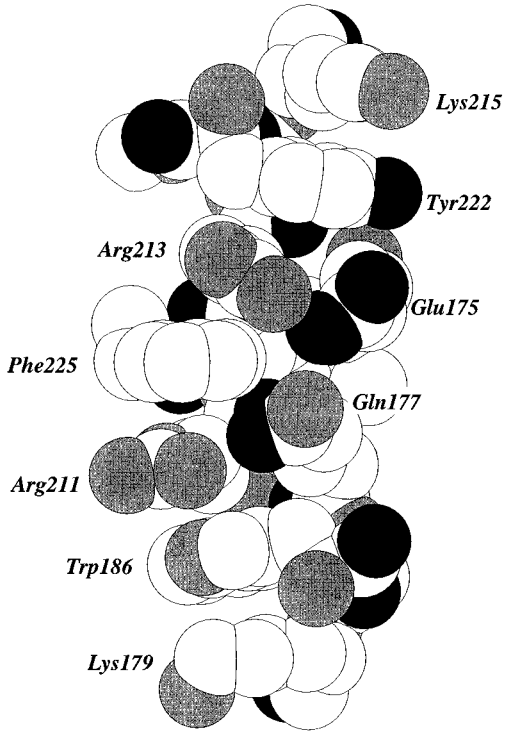


Figure 6. Extended cation−π interactions seen in the human growth hormone receptor extracellular domain.[136] Coordinates are taken directly from the Brookhaven Protein Data Bank file 3HHR, and the figure is based on the view first presented by Wilson.[135] Code: carbon, white; nitrogen, light gray; oxygen, dark gray. Hydrogens are omitted, thus, e.g. the N of a Lys is actually NH_3^+, the N of an Arg is actually NH_2, etc.

of UMP/CMP kinase with a bisubstrate inhibitor.[134] A spectacular example of a series of cation−π interactions involving both Arg and Lys was recognized by Wilson in the erythropoietin receptor extracellular domain.[135] Wilson also noted very similar motifs in previously determined structures of the human growth hormone receptor extracellular domain[136] and human prolactin receptor extracellular domain.[137] All these structures contain several aromatic and cationic side chains from different strands of the protein interdigitated to form an extended array of cation−π interactions. The particular array from the human growth hormone receptor is shown in Figure 6. From top to bottom, one sees Lys-Tyr-Arg-Phe-Arg-Trp-Lys in a more or less continuous stacked array. Note that other more conventional interactions are also evident: a hydrogen bond between Arg211 and Gln177; and a salt bridge between Arg213 and Glu175. Here, as in many other examples cited below, cation−π interactions and more typical forces are seen to work in concert. A similar, but less extended, interaction is seen in α-amylase inhibitor, in which a highly conserved sequence, Trp-Arg-Tyr, displays an extended stacking interaction in crystal structures,[138] and in solution as determined by NOE experiments.[139]

Another common manifestation of cation−π interactions is an alteration of side-chain pK_a values. Protonation might be expected to be favored if the resulting cation experiences special stabilization, and this has been seen in several instances. For example, a lysine-phenylalanine interaction has been observed in an 18-residue peptide.[140] Of the three Lys in the peptide, only one can interact with the Phe, and it shows an anomalous pK_a. Histidine can also interact with aromatic residues in its protonated form. In particular, such a cation−π interaction is believed to be the cause of an elevated pK_a for a histidine in barnase that has been observed by various methods. Loewenthal et al. first observed a pH dependence of Trp fluorescence intensity with an inflection point at pH 7.75.[141,142] Mutation studies showed a His is essential in quenching the Trp. Using double mutant cycles and direct measurement of the His pK_a's, they determined that the His^+−Trp interaction is worth 1.4 kcal/mol, while the neutral His−Trp interaction is worth 0.4 kcal/mol relative to solvation in water.

Additionally, the cation−π interaction can contribute to helix stability. Shoemaker first observed a pH dependence of helical stability involving a Phe−His pair.[143] A more systematic study of 17 residue peptides demonstrated that the pH, the distance between the interacting residues, and the aromaticity of the Phe residue are all critical to the helix stability.[144] This interaction has also been used in peptide design. For example, a dibenzofuran with two flanking histidine residues has been used successfully in small peptides at low pH's as a β sheet nucleating agent.[145]

B. Binding of Acetylcholine and Related Ligands

1. Acetylcholine Esterase

Acetylcholine esterase plays a crucial role at cholinergic synapses by hydrolyzing acetylcholine (ACh) to choline plus acetate, thereby terminating synaptic transmission. It is much studied as the target of potent toxins of the carbamoyl ester and fluorophosphonate families, and, more recently, as a target of therapeutic agents for Alzheimer's disease. It has also played a crucial role in efforts to establish the biological relevance of the cation−π interaction.

Although most discussion of the esterase prior to 1990 described an "anionic subsite"—the feature that bound the cationic quat of ACh—there were some early efforts that did not support this view. An especially prescient work was that of Höltje and Kier, which invoked a simple theoretical model emphasizing electrostatic interactions to suggest that an aromatic ring could play the role of the "anion" at the binding site.[146] Important studies on the esterase by Cohen also downplayed the "anionic" nature of the binding site, recognizing that it must be somewhat hydrophobic and suggesting the term "trimethyl" subsite.[147−149] Studies such as these coupled with our observations on cyclophane binding of quats led us to propose in 1990 that cation−π interactions would be important in binding ACh.[55]

The breakthrough came in 1991 with Sussman's determination of the acetylcholine esterase structure to 2.8 Å resolution.[150] This beautiful structure has provided valuable insights on many fronts, not the least of which is the cation−π interaction. The active site lies at the bottom of a deep, narrow gorge, a substantial portion of which is lined by 14 conserved aromatic residues. At the active site, the quat of ACh is in contact with the side chain of the highly conserved Trp-84. The esterase remains to date the

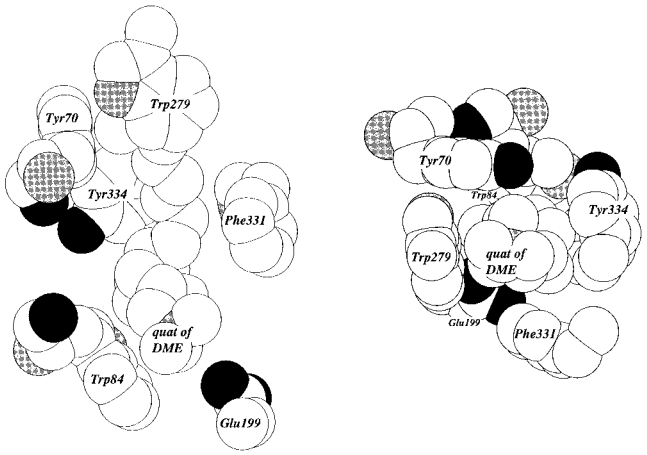


Figure 7. Two views of the acetylcholine esterase structure complexed to the bisquat decamethonium (DME).[151] Coordinates are taken directly from the Brookhaven Protein Data Bank file 1ACL. Code: carbon, white; nitrogen, light gray; oxygen, dark gray. Hydrogens are omitted. Left: Side-on view of the aromatic gorge. The active site region is at the bottom, showing the quat of DME that is thought to mimic the quat of ACh. Right: View looking down the aromatic gorge from the outside of the protein, showing the other quat of DME complexed to the remote anionic site, Trp279.

only natural ACh binding site whose structure has been determined.

Subsequent studies confirmed and expanded upon the initial findings of the X-ray structure. Structures were determined for the esterase with bound ligands such as edrophonium, which is used chemically to diagnose myasthenia gravis; tacrine, a promising therapeutic for Alzheimer's disease; and decamethonium ($Me_3N^+(CH_2)_{10}NMe_3^+$), a potent cholinesterase inhibitor.[151–154] Two views of the decamethonium structure are shown in Figure 7. The active site quat is in contact with Trp84. Also nearby is Glu199, which one would expect to contribute to binding, although mutation studies suggest that it is not directly involved in binding.[155] Phe331 typifies the aromatics that line the gorge, although all such residues could not be shown without obliterating the ligand. The second quat of decamethonium makes a close contact with Trp279 and is also near Tyr334. These results establish that the so-called "remote anionic site", like the primary anionic site, is, in fact, a Trp.

A large number of more recent biochemical studies confirm the important role of aromatic residues in the esterase proposed on the basis of the crystallographic data. Highlights include: mutagenesis studies which confirm that Trp-84 is a "critical element in the active center";[156,157] and that it "constitutes the classical anionic subsite";[158] affinity labeling studies that confirm the role of Trp-279 in the remote anionic site;[159] and studies of the snake neurotoxin fasciculin that suggest cation–π interactions between two arginines of the toxin and aromatics of the esterase.[160]

2. McPC603

Another crucial early structure was that of the antibody Fab McPC603, which binds phosphocholine ($Me_3N^+CH_2CH_2OPO_3^{2-}$).[161,162] In addition to major binding contacts between arginine side chains and the phosphate group, the closest contacts to the quat are made by three aromatics, especially Trp-107H. Taken in combination with the esterase structure, these results suggest that tryptophan may be especially prominent at quat binding sites. This would be consistent with the theoretical studies cited above, which suggest Trp should provide the most potent cation–π binding sites. Note that the cobra venom phospholipase A_2 is 80% similar to the McPC603 antibody, and so it too employs cation–π interactions in binding of *n*-alkylphosphocholines.[163,164]

The binding of phosphocholine by McPC603 also involves carboxylates. Both an aspartate and a glutamate are near the quat, but not as near as the three aromatics.[55,161] Still, one might expect the ion pair interactions between the quat and these carboxylates to be longer range, and so certainly they contribute to binding. It must be emphasized cation–π binding can work in concert with more conventional interactions, and there is evidence that this is so in McPC603, the acetylcholine esterase, and the nicotinic and muscarinic acetylcholine receptors (see below).

3. Nicotinic Acetylcholine Receptor (nAChR)

The nicotinic acetylcholine receptor (nAChR) is the longest-known, best-characterized neuroreceptor,[165–169] and it provides some of the strongest support for cation–π interactions at ACh-binding sites. The receptor is the prototypical ligand-gated ion channel—ACh binds to the receptor, inducing a conformational change that opens an ion channel that is contained within the protein. Nicotine is a full agonist for this receptor, and certainly the nAChR plays an important role in nicotine addiction. The receptor consists of five homologous subunits in an $\alpha_2\beta\gamma\delta$ stoichiometry (Figure 8). There are two agonist binding sites, thought to be primarily associated with the α subunits, although many workers feel the binding sites are at subunit interfaces, involving α/γ and α/δ. Although only a low-resolution structure is available,[168,169] a large number of less direct chemical and spectroscopic methods have produced considerable amounts of information about the ACh binding site.

Pioneering work by Karlin established contributions to the agonist binding site from a region near Cys192-Cys193 of the α subunit—a region that is rich in aromatic residues.[170] Then, in ground-breaking work, Changeux and co-workers found that the photoaffinity reagent [^{3}H]DDF (*p*-(dimethylamino)-benzenediazonium fluoroborate) labeled many α-subunit aromatic amino acid residues (Figure 8).[166,171–173] These residues are believe to directly contribute to binding the charged portion of the agonist since the reactive functional group on DDF is a positively charged diazonium salt. Many of these aromatic residues are also independently labeled by other reagents. Abramson, for example, found that lophotoxin binds very selectively and covalently to Tyr190.[174] In an especially telling experiment from Cohen, Tyr93 was alkylated by radioactive acetylcholine mustard in its aziridinium form.[175] Because carboxylates are expected to react substantially faster

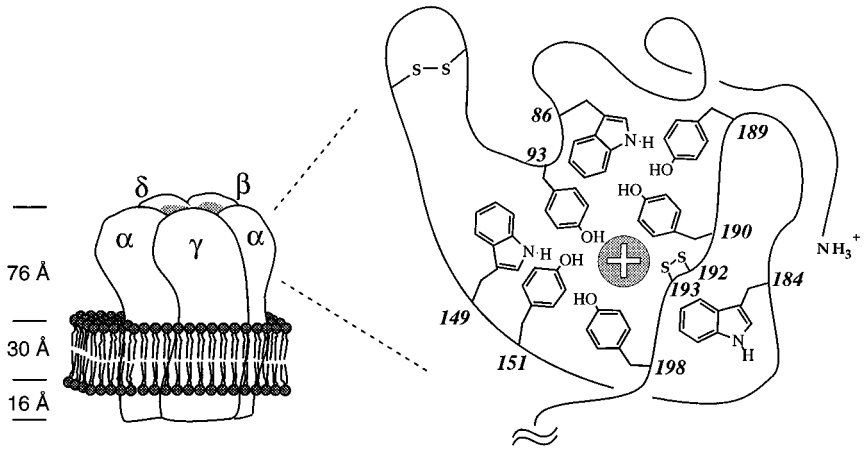


Figure 8. Two views of the nAChR: (left) overall layout of the receptor according to the results of Unwin[168,169] and (right) highly schematic view of the agonist binding site (α subunit) showing the many aromatic residues thought to contribute to defining the site. The drawing is adapted from one first presented by Changeux.[166,204]

with the aziridinium ion than Tyr, the labeling of Tyr93 with high efficiency suggests that there are no anionic residues near the trimethylammonium binding region. Nicotine[176] and *d*-tubocurarine (a cationic competetive antagonist) also labeled several aromatic residues on both the α subunits and on the adjacent γ and δ subunits.[177] These affinity-labeled aromatic residues are highly conserved in the homologous positions in all known α subunits of nicotinic receptors, but not in the β, γ, and δ units.

A great deal of supporting evidence for a crucial role for aromatic residues at the agonist binding site of the nAChR has been gathered from many different sources. Extensive studies of peptides that correspond to crucial regions of the receptor confirm an important role for aromatics.[178−184] These studies and studies of the intact receptor establish that bungarotoxin binding—a hallmark of nicotinic receptors—is strongly influenced by aromatic residues.[185,186] Conversely, a cationic arginine residue plays a crucial role in the binding of various toxins such as erabutoxin, presumably through interactions with aromatics on the receptor.[187,188]

Several spectroscopic methods have been applied to the nAChR. Using receptor peptide fragments, Fraenkel used NMR NOE methods to establish close contacts between the quat of ACh and Trp184.[189] Other, related ligands also interact with this tryptophan. Interestingly, NMR indicates that ACh undergoes a conformational change on binding, displaying a more compact geometry at the binding site than when free in solution.[190,191] FT-IR methods have been applied to the complete, intact receptor. Using FT-IR difference methodologies, Baenziger[192,193] observed a measurable change before vs after exposure of a thin film of receptor to agonist. Ligand binding led to a new stretching vibration at 1516 cm^{-1}, which was interpreted to indicate a conformational change involving a tyrosine residue.

Extensive mutagenesis studies have confirmed and expanded upon the affinity labeling and spectroscopic studies. Early studies by White and Yellen of tyrosines 93, 190, and 198 confirmed an important role for these residues,[194−196] and these results were further substantiated by studies from Sine and our own group, which indicated that aromatic amino acids were strongly preferred at these sites.[197−199] More recent studies have also identified a highly conserved aromatic residue on the γ/δ subunits that may also contribute to agonist binding.[200]

While a huge body of evidence establishes a crucial role for a large number of aromatic residues at the agonist binding site of the nAChR, there is, in fact, no proof yet for a cation−π interaction. Extensive studies of the Tyr93, Tyr190, and Tyr198 sites using the *in vivo* suppression methodology for unnatural amino acid incorporation indicate that these tyrosines are probably not the primary cation−π sites.[198,199] Of course, given all of the above results, one might suspect one of the several tryptophans to be primarily responsible for cation−π interactions. These sites have been less extensively probed by mutagenesis methods, although such work is in progress.[201]

Also, more recent studies have identified an anionic residue that appears to contribute to the agonist binding site. It is not on the α subunit, but rather is on γ/δ. Clever studies by Karlin place it < 10 Å from the binding region, and mutagenesis studies suggest it does contribute to binding.[202,203] It is surprising, then, that apparently none of the affinity labeling agents ever labeled this site. Nevertheless, the implication is that at the nAChR, as at several other sites discussed here, aromatic and anionic residues work together to bind cationic ligands.

Note that the nAChR is the prototype of the superfamily of ligand gated ion channels, which also includes glycine receptors, $GABA_A$ receptors, serotonin ($5HT_3$) receptors, and the more remotely related family of glutamate receptors (NMDA, AMPA, and kainate receptors).[204,205] For the most part these other systems have not been as well studied as the nAChR, but there is some evidence that aromatic residues play an important binding role in some of these structures.[206−211] An interesting example concerns the glycine site of the NMDA receptor, where a cationic group in the receptor site is proposed to interact with a carboxylate on kynurenic acid antagonists. However, workers at Merck found that the carboxylate can be replaced by an aromatic ring, presumably replacing an ion pair interaction with a cation−π interaction.[212] A similar replacement has

been used in inhibitors of N-succinyl-LL-diaminopimelate aminotransferase.[213]

4. The Muscarinic ACh Receptor and Other G Protein-Coupled Receptors

The G protein-coupled receptors (GPCR) are an important class of membrane-bound proteins involved in many signal transduction pathways. These seven-helix receptors bind a wide range of cationic amines such as acetylcholine (the muscarinic receptor), dopamine, epinephrine, and serotonin, as well as a large number of peptidic ligands such as bradykinin and tachykinin.

Although no high-resolution structure is available, a number of models for these seven-helix receptors have been built starting from the low-resolution structures for bacteriorhodopsin and rhodopsin, the latter being a member of the GPCR class.[214,215] Hibert *et al.* modeled 22 GPCR placing the cationic group of ACh, dopamine, or serotonin next to a conserved Asp carboxylate located at the bottom of the putative binding cleft.[216–218] The authors found three highly conserved aromatic residues surrounding the ion pair. Later modeling studies implicated two additional tyrosine residues on helix 7 that can contribute to ligand stabilization.[217] Similar modeling studies of muscarinic agonists showed that the cationic group is in close contact with an anionic Asp and two aromatic residues.[219] This study also proposed an aromatic gorge located above the binding site that facilitates the entry of the ligand through cation–π stabilization. These binding site features are quite general to this class of receptors. A model of the D2 dopamine receptor shows that the NH_3^+ of dopamine interacts with an Asp and Phe at the binding site.[220] Interestingly, a differently constructed 3D model also implicated three aromatic residues that contribute to ligand binding. Donnelly et al. modeled their receptor on the basis of Fourier transform analysis of the sequence alignment of 59 aminergic GPCR and the projection of the helix arrangements of bovine rhodopsin.[221,222] Their model reproduced two of the aromatic residues in Hibert's model and found that the third residue is on helix 7 rather than helix 6. These models identifying one or more key aromatic residues working with the highly conserved aspartate are consistent with earlier speculation by Pearce that suggested a crucial role for the aspartate and a highly conserved tyrosine.[182]

These modeling studies of GPCR suggest but again do not prove the involvement of aromatic residues in cationic ligand binding. Still these models have served as useful tools in providing guidelines for molecular studies of specific residues. For example, mutagenesis and binding studies of the V1a vasopressin receptor have since shown that an Arg of a protein ligand is crucial to the high affinity binding, and the agonist selectivity can be modulated by mutation of a key tyrosine to Asp or Phe.[223] In binding of both neuropeptide Y and the nonpeptide antagonist BIBP 3226 by the Y1 receptor, modeling suggested and mutagenesis confirmed key roles for three aromatic residues, including a cation–π interaction of a guanidinium group of the antagonist with a Phe on the receptor.[224] A similar interaction is believed to contribute to the binding of CP96345 to the neurokinin-1 receptor.[225] Molecular modeling on the dopamine D2 receptor suggested mutagenesis studies that eventually implicated two aromatic residues that are important in binding.[226] Recently, a completely synthetic receptor for biogenic amines has been developed, using a combination of ion pair and cation–π interactions analogous to those proposed in the GPCR.[227]

As in other cases discussed above, the binding of cations by the GPCR class may involve a combination of cation–π interactions and conventional ion pairing, in this case to the highly conserved aspartate residue. There has been considerable debate as to the relative importance of these two effects, highlighting the difficulties in dissecting complex molecular interactions.[228–230]

C. Other Protein–Ligand Interactions

1. Alkylamine Dehydrogenases

The first suggestion of possible cation–π interactions in the alkylamine dehydrogenases came from a 6.0 Å resolution crystal structure of the ligand-bound trimethylamine dehydrogenase.[231] The crystalline dehydrogenase soaked in tetramethylammonium chloride or trimethylamine (TrMA) incorporated these ligands in a hydrophobic site that is rich in aromatic residues (Tyr and Trp). Because of the high sequence homology between the trimethylamine and the dimethylamine dehydrogenase, a model for the dimethylamine (DMA) dehydrogenase binding site was constructed[232] using the high-resolution structure of the TrMA dehydrogenase.[233] The only difference found between the two sites was the replacement of a Tyr in the TrMA dehydrogenase by a Gln in the DMA dehydrogenase. In fact, the dimethylamine dehydrogenase positions the glutamine to hydrogen bond with the proton, while the remaining aromatic residues bind the methyl groups as in the trimethylamine binding site.

Another interesting cation binding property was seen in methylamine dehydrogenase. In one of the binding sites, termed a type II site, the ion binding preference follows the Eisenman class IV sequence (low field strength selectivity), $K^+ \approx Rb^+ \approx Cs^+ > Na^+ > Li^+$.[234] This selectivity is suggestive of an aromatic binding site, as the balance between desolvation and cation–π interactions can select for potassium ions to bind the benzene ring in water (see K^+ channel section below).[19] The authors suggest that selectivity at the type II site may result from a cation–π interaction with the tryptophan tryptophylquinone cofactor (TTQ) or another enzyme bound aromatic group.

2. SH2 domains

The binding event in many cell surface receptors results in the phosphorylation of the receptor protein at tyrosine sites. Following this transformation, these phosphorylated tyrosines are recognized by downstream proteins containing SH2 domains. The SH2 (src homology 2) regions are small modular domains of ~100 amino acids that mediate protein–protein interactions by recognizing and binding spe-

cific sequences containing the phosphorylated tyrosines. Initially discovered as sequence homology domains shared by a number of oncogene products, they communicate the phosphorylation states of the signal transduction proteins.

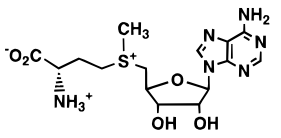
The crystal structures of the *v-src* oncogene product complexed by two pentapeptides containing phosphorylated tyrosine have been solved at 1.5 and 2.0 Å resolution.[235−237] Within the binding cleft, two positively charged residues, Arg-155 and Lys-203, interact directly with the aromatic ring of the phosphotyrosine while forming hydrogen bonds with proximal oxygens. The guanidinium of the Arg points into the aromatic ring, and the terminal amino group of the lysine residue is 3.8 Å below the edge of the ring, within the range to form a cation−π interaction. The arginine residue in particular is highly conserved among the known SH2 domains. Other crystal structures of SH2−domain−phosphotyrosine−peptide complexes showed similar interactions between the aromatic ring and the positively charged residues. For example, in the crystal structure of the peptide-lck SH2 region,[238] or in the ZapNC tandem SH2 domain,[239,240] the same interaction was observed between the active-site arginine and the aromatic ring of the peptide.

Additionally, NMR experiments have confirmed the interaction between the aromatic ring and the charged residues in SH2 domains. In phospholipase C-γ1 (PLCC), NOE and ^{15}N and ^{13}C NMR shifts and relaxation studies of the arginine showed that the guanidinium group interacts with the pTyr ring.[241] Another NMR-derived structure showed a His to be perpendicular to the phosphotyrosine ring in the Shc SH2 domain.[242,243]

D. Cation–π Interactions in Catalysis

1. S-Adenosylmethionine (SAM)

Research in the Dougherty lab showed that aromatic synthetic receptors can catalyze the alkylation of quinoline derivatives and the dealkylation of sulfonium ions.[244−246] The dealkylation reaction is reminiscent of methylation by *S*-adenosylmethionine (SAM) in biological systems. The cationic, sulfonium compound SAM can methylate any of a broad range of structures, including nucleic acids, proteins, sugars, and C=C double bonds of steroids and lipids. The similarity of the two systems led us to propose that cation−π interactions might be important in reactions involving SAM.[244,245]



SAM

Subsequently, the crystal structure of a cytosine-DNA methyltransferase was reported.[247] It reveals a van der Waals contact between the S^+-CH_3 unit of SAM and the π face of a Trp residue, in a favorable alignment for catalysis assisted by cation−π interactions.[1] Analysis of many methyltransferase sequences reveals a conserved motif that is "unusually rich" in aromatic residues and has been proposed to be involved in binding SAM.[248] Within this motif, the binding of SAM was found to tolerate a tyrosine to phenylalanine mutation, but not a valine mutation.[249] This certainly suggests that the aromatic side chain is integral to the SAM binding. In this case, the authors specifically propose that the tyrosine binds the sulfonium ion through cation−π interaction.

2. Steroid Synthesis

Squalene cyclization is a crucial step in the steroid biosynthetic pathway. The cyclization is postulated to be initiated by the protonation of an epoxide, which leads to a cationic cascade reaction. This cyclizes the ring and provides a dramatic demonstration of enzymatic catalysis, when in one step seven stereocenters are generated while a carbocation traverses the polyene backbone. Thus, it was suggested by Johnson that the cyclase contains a series of anionic sites which guide the cation generation.[250] However, the only available anionic functional groups in proteins are carboxylates, and the carboxylates might be expected to react irreversibly with a carbocation to form an ester.

Subsequently, genes encoding oxidosqualene-lanosterol cyclases from several organisms were sequenced.[251−253] Tryptophan and tyrosine residues are unusually abundant in all of these predicted amino acid sequences.[251] In particular, 16 of the 17 Trp residues and 34 of the 40 Tyr residues on the *S. cerevisiae* cyclase sequence are found at identical locations within the *C. albicans* sequence. These results suggested that the aromatic π electrons may play an integral role in the cyclase activity.

Consistent with this notion is the appearance of a recurring sequence in the cyclase enzymes termed the QW motif. This sequence is rich in aromatic residues and occurs in all cyclases.[252] In the rat cyclase, for example, the motif was found to repeat six times, and overall, the cyclase contains "a disproportionally higher number of aromatic residues that are completely conserved, whereas the negatively charged Asp and Glu resides are less highly conserved".[253] These observations, combined with Dougherty's demonstration of aromatic stabilization of cationic transition states, led both Poralla and Griffin to propose specifically that the "anionic sites" in Johnson's mechanism are, in fact, aromatic residues.[251,252,254] Building on this, Griffin has recently designed novel pyridinium-based inhibitors of the cyclase that resemble the type of guests that are especially well-suited to cation−π interactions in cyclophane receptors.[255]

E. Ion Channels

1. K^+ channels

a. The Pore Region. Potassium channels can be much more ion selective than ligand-gated channels such as the nAChR discussed earlier, with selectivities for K^+ over Na^+ perhaps as high as 1000:1. Little structural information was available for this class of proteins until the successful cloning and sequencing of the *Shaker* gene of *Drosophila*, which codes a voltage-gated K^+ channel.[256] Structurally, voltage-

gated channels generally consist of four subunits that assemble around the channel pore. Each subunit has five hydrophobic transmembrane segments and one transmembrane segment with several positively charged residues that apparently serve as the voltage sensor. A short segment, called the SS1 and SS2, H5, or pore region has been identified to define an important component of the ion channel. Most strikingly, the pore region is rather hydrophobic in nature, and contains a number of highly conserved aromatic residues.

Subsequently, a second general class of K^+ channels was revealed, the inward-rectifier K^+ channels. These contain a pore sequence that is highly homologous to that of the voltage-gated channels. They differ in having only two transmembrane regions, which flank the pore region.

Sequence alignment of all the cloned K^+ channels revealed a homologous "signature sequence", TXXTXGYG in the pore region. In particular, the highly conserved Gly-Tyr-Gly (GYG) sequence appears to be essential for ion selectivity. This result led Heginbotham and MacKinnon to propose that perhaps cation–π interactions are responsible for establishing ion selectivity in K^+ channels.[257] Two independent computer models of K^+ channels both suggested the involvement of the aromatic residues in ion selectivity. Bogusz *et al.* generated eight possible pore models based on analysis of a family of 12 antiparallel β-barrel proteins.[258] All of the models project many aromatic side chains into the pore. Citing the cation–π interaction, they suggested that these residues contribute to ion selectivity in this otherwise lipophilic region. Another model by Durell and Guy implicated the Tyr residue in the highly conserved GYG triplet.[259] Specifically, they proposed that this Tyr forms a tetrameric cage with the corresponding Tyr's on the other subunits. This cage serves as the narrowest region of the pore and is integral to ion selectivity. It should be noted that other computer models have been generated which do not implicate cation–π interactions.[260]

Kumpf and Dougherty tested these proposals in a theoretical study of the interaction of simple cations with benzene both in the gas phase and in water.[19] Combining gas-phase binding energies determined from ab initio quantum mechanical methods with Monte Carlo methods to model aqueous solvation effects, they evaluated the potential for selectivity of a cation–π site in aqueous media. As noted above, the gas-phase binding sequence is $Li^+ > Na^+ > K^+ > Rb^+$. In water, however, a dramatic reordering is seen, and the affinity of a cation–π site follows the order $K^+ > Rb^+ \gg Na^+, Li^+$. This is qualitatively the same sequence seen in K^+ channels.

Such a result does not prove that cation–π interactions are involved in the channel, only that it is physically plausible to create a selective channel using cation–π interactions. There are certainly other mechanisms (anionic side chains, neutral oxygens from side chains or backbone carbonyls, etc.) that are plausible and have been seen in other channels.[261,262] It has even been proposed, on the basis of aqueous gel sieving chromatography, that just a generic hydrophobic site could select for K^+, even without cation–π interactions.[263] Continued experimental investigation has yet to resolve the issue. Heginbotham and MacKinnon showed that mutating the crucial pore tyrosine to a valine in a voltage-gated channel produced a channel with somewhat altered properties but still at least some selectivity for K^+ over Na^+.[264] In contrast, studies from our lab[265] and from Gaber and co-workers[266] on inward-rectifying channels suggest an absolute requirement for an aromatic residue at this site in order to achieve the highly selective behavior of the natural K^+ channels. These studies illustrate the challenge of establishing detailed interactions in ion channel proteins.

b. TEA Blockade of K^+ Channels. Mutagenesis studies showed that the cation–π interaction is likely to be important in another feature of voltage-gated K^+ channels: blockade by organic cations such as tetraethylammonium (TEA). Site-directed mutagenesis identified a Tyr at position 449, near the mouth of the pore, which is crucial to TEA binding.[257,267–269] In fact, the free energy of binding is linearly dependent on the number of subunits containing tyrosine at this particular site. Across a range of related K^+ channels, those with high TEA affinity have a Tyr at this crucial site, while low TEA affinity channels, like *Shaker,* have a nonaromatic residue such as threonine at this position. More importantly, high-affinity binding to TEA can be induced in *Shaker* by mutation of this residue to Tyr.[257,267]

2. Guanidinium Toxins Binding to Na^+ Channels

Similarly, sequence comparison between the high- and low-affinity toxin binding Na^+ channels implicates aromatic residues. Guanidinium toxins tetrodotoxin (TTX) and saxitoxin exclusively block sodium channels, but have varying affinities to different subclasses. The cardiac sodium channels are less TTX sensitive than the brain or skeletal muscle Na^+ channels. Only two residues in the pore region are different among these channels, with the crucial distinction arising from a Phe/Cys pair. Satin et al. mutated a cysteine in cardiac channels to Tyr or Phe and found that the TTX blocking efficiency increased by 730-fold, an increase of 3.9 kcal/mol in energy.[270] These mutant channels resembled (and even surpassed the affinity of) those of the brain and skeletal muscle channels. The authors specifically propose that the aromatic residue binds to the TTX in the brain and skeletal muscle channels through a cation–π interaction with an arginine of the toxin. Conversely, the mutation of Tyr to cysteine in the skeletal muscle channels, or the mutation of Phe to cysteine in the brain channels reduced the TTX sensitivity substantially.[271–273] While modeling studies have suggested a prominent role for hydrophobic effects in the binding of such toxins,[274,275] detailed experimental studies reveal a strong electrostatic component.[276]

F. Additional Examples

Cation–π interactions have also been suggested as important recognition elements in ligand binding by other proteins. For example, in trypanothione reductase a tryptophan is believed to make cation–π

contact with the ammonium group of trypanothione. In fact, the selectivity of the trypanothione reductase can be switched to bind glutathione by only two mutations, one of which converts a Trp to an Arg.[232,277−279] Cation−π interactions may also be important in horseradish peroxidase, where an active site arginine is essential to the aromatic donor binding, and an adjacent tyrosine residue appears to also contribute to binding.[280] In factor Xa, an important serine protease, the S4 subsite has no acidic residue, and is proposed to be formed by three π faces of aromatic residues, which bind a cationic group on enzyme inhibitors.[281] A Tyr residue is found in the glutamine synthetase ammonium substrate binding site, where the aromatic side chain is shown to interact with Cs^+ and Tl^+ ions in the crystal structures.[282] Similarly, a Cs^+ soaked into a crystal of rhodanese binds to a tryptophan residue.[283]

Recently, the crystal structure of the DNA glycosylase AlkA reveals a large hydrophobic cleft rich in aromatic residues in the enzyme active site. The common feature of the base recognized by the enzyme is a positive charge formed by alkylation, suggesting cation−π interactions are important in binding.[284] The crystal structures of the protein−chromophore complex of neocarzinostatin and its apoprotein form have been determined at 1.8 Å resolution. It was suggested that a sugar amino group of the chromophore is protonated and interacts with a Phe ring in the active protein.[285] Sulfoxide inhibitors of the liver alcohol dehydrogenase-NADH complex bind so as to position the positively charged S of the sulfoxide adjacent to a phenylalanine side chain.[286] A conserved tyrosine may contribute to the allosteric Na^+ binding site of a class of serine proteases that includes thrombin.[287]

Another structure for which aromatic functionality has been suspected to play a crucial role is P-glycoprotein, the multidrug resistance transporter that appears to play a seriously detrimental role in cancer chemotherapy. This is a prototype of a large class of membrane proteins containing 12 transmembrane regions. Substrate binding occurs within the transmembrane region[288] (reminiscent of the GPCR discussed above), and this region is especially rich in aromatic residues.[289] Note that this protein transports cationic and neutral molecules, but not anionic structures, consistent with expectations of a site comprised of many aromatics.[290] Other structures in which aromatic amino acids are also especially prominent in general include: antibody binding sites,[291] consistent with the varying roles aromatics can play in binding; and regions of proteins that are near membrane interfaces,[292,293] perhaps indicative of a cation−π interaction with the choline head groups of the lipids.

V. Conclusions

Cation−π interactions have now been characterized in a wide range of contexts. Fundamental gas-phase studies, both experimental and theoretical, established the cation−π interaction to be among the strongest of noncovalent binding forces. For prototype systems, a simple electrostatic model rationalizes major binding trends, and also provides useful guidelines for understanding more complex structures. Crucial insights into the nature of cation−π interactions have been provided by studies of artificial receptors, especially in aqueous media. These complexes established that an organic binding site composed primarily of aromatic groups can compete with full aqueous solvation and move an organic cation out of water and into a hydrophobic environment. The biological implications of such findings are profound.

It is now clear that cation−π interactions have a prominent position among the various noncovalent binding forces that Nature uses to assemble the molecules of life. Across a wide range of structural types, cation−π interactions have been documented, or at least strongly implicated, to be important in protein stuctures and protein−ligand interactions. An important implication of the cation−π interaction and the related "polar-π" interactions[43−47] is that Phe, Tyr, and Trp should not be considered simply "hydrophobic" amino acids. They are in fact distinct from the conventional hydrophobic residues such as Val, Leu, and Ile. Because of the dual nature of benzene—hydrophobic yet (quadru)polar[38]—aromatic amino acids are expected to play a unique role in protein structure and function.

VI. Acknowledgments

D.A.D. thanks the many outstanding graduate students and postdocs who have contributed to our work on cation−π interactions over the years. The work at Caltech has been supported by the Office of Naval Research, the National Institutes of Health, and Zeneca Pharmaceuticals, to whom we are grateful.

Note Added in Proof

A number of publications describing cation−π interactions have appeared since the original submission of this manuscript. Theoretical studies indicate an important catalytic role for cation−π interactions in the acetylcholine esterase.[294] An extensive study of cation−π-driven complexation to neutral calixarene hosts in chloroform has appeared.[295] Several studies on drug−receptor interactions have appeared, including the following: high affinity, ultraselective antagonists of the δ opioid receptor;[296] a possible replacement of a carboxylate−cation interaction with a cation−π interaction in a key bacterial enzyme;[297] and a possible cation−π interaction to a Mg^{2+} ion in the HIV integrase.[298] The latter paper also reports ab initio calculations of benzene and catechol binding to Mg^{2+} and Mn^{2+}. Several further examples of cation−π interactions in proteins have also appeared. These include two articles describing (i,i+4) cation−π interactions in α helices;[299,300] an especially prominent role for Arg−Trp interactions in surface adhesive peptides;[301] cation−π interactions in the human butyrylcholinesterase;[302] further, compelling evidence concerning alkylammonium ion specificity in trimethylamine dehydrogenase;[303] and several possible cation−π interactions in the nonclassical homeodomain from rat liver LFB1/HNF1 transcription factor.[304]

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