Available online at www.sciencedirect.com
ScienceDirect
Coordination Chemistry Reviews 250 (2006) 2918–2928
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Reflections on the construction of anion receptors Is there a sign to resign from design?

Franz P. Schmidtchen *

Department of Chemistry, Technical University of Munich, Lichtenbergstrasse 4, D-85747 Garching, Germany

Received 18 January 2006; accepted 21 July 2006
Available online 31 July 2006

Contents

Abstract

The principles and conceptual basis of the design of molecular hosts for anions is examined. Starting from general considerations on the fundamentals of supramolecular binding and selectivity the role of the underlying model is illuminated. Turning to an agenda to be followed in the construction of concrete anion hosts the aspects of function, the guest to be bound, the competition situation and the strategic feasibility are discussed. Finally, arguments to include various facets of association entropy are presented that were unraveled by recent experimental studies. As a conclusion, the necessity to consider entropic influences in host design is emphasized. Although this adds to the complexity of the design task an optimistic prospect for success is voiced in view of recent progress in computational prediction and the support from experimental entropy data.

Keywords: Molecular recognition; Host–guest design; Anion receptor; Selectivity; Association entropy

1. Introduction

At first sight the construction of artificial hosts for anions seems so easy and straightforward. Putatively, all that is needed is to introduce a molecule having an intrinsic affinity for a species carrying a negative charge. There are legions of options. In this sense a simple metal cation, say Na^+ would be an adequate solution to furnish a one on one associative process with chloride anion. Inserting the charge and ionic radii into Coulomb's law leads to a tremendous binding constant of $>10^{300}$, however, in free space (entropic influences do not change the basic argument in this regime). This admittedly rather odd example demonstrates clearly that binding itself should not present a central problem. What then makes the construction of anion receptors such a demanding task that the development of the field lagged well behind the corresponding investigations of cation receptors, and even today the most sophisticated examples still do not live up to the standards set by the natural counterparts? Obviously, there are hidden obstacles and camouflaged pitfalls obstructing the ready translation of our wishful thinking into fully functional host compounds. Traditional guidelines appear to provide insufficient wisdom for designing [1] abiotic receptors of utility in concrete technical applications. We may even face the necessity to re-think and modify fundamental principles in host design in order to make the much desired progress.

* Tel.: +49 89 289 13338; fax: +49 89 289 14698.
E-mail address: Schmidtchen@ch.tum.de.

0010-8545/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ccr.2006.07.009

Looking back on more than 30 years of anion receptor chemistry [2] in this author's perception quite a number of honest attempts at host design failed because of basic misconceptions or careless or unintentional, yet prejudiced interpretation of the experimental observables. Especially novices to the field might thus be confused and left at risk to violate tacit presumptions and fail to pay the due respect to the intrinsic limits of the concepts followed or the physical methods used.

This article presents a personal view on the prerequisites of the design of anion receptors enriched with some examples from 30 years of moderately successful attempts [3] to excavate and illuminate the hidden base of anion recognition.

2. The goal

The ultimate purpose of host design emerges from the basic strive of chemists to be in command of the chemical and physical behavior of anions at will. In order to achieve this, the anion (the guest) has to be positioned into a non-isotropic, well-structured environment (i.e. the host) that will allow or favour only much fewer and potentially altered responses to chemical or physical stimuli than possible in isotropic solution. As a result of this association aggregate species are formed (the host–guest complexes) that may vary in their subtle appearance subject to the disposition and mutual "stickyness" of the component parts (substructures) of the interacting partners.

Creation of selectivity that extends beyond the mere binding capacity and allows molecular recognition is the ultimate goal, however, also the true challenge in host design. As a corollary, selectivity could serve as a yardstick to asses the quality of a particular design attempt and document the progress. Unfortunately, the true meaning and usage of the term is quite ambiguous [3,4]. A close inspection confirms that selectivity is by no means an absolute measure, but is intimately tied to a function. In the context of host design this necessitates the definition of a purpose. Additionally, environmental conditions (physical variables like temperature, pressure, but also the solvent, the nature and quantity of competing species or the presence of phase boundaries) greatly influence the selectivity pattern rendering the construction of receptors of universal specificity impossible.

Selectivity of function comes in two versions (see Fig. 1) [4,5]: in the thermodynamic domain selectivity is expressed in equilibrium binding of the various competing substrates in a time independent fashion. Affinity and initial concentrations of the individual species determine the observable preference and define the discrimination pattern. Some important applications like ion sequestration or two-phase extractions build upon such thermodynamic selectivity. The majority of applications and particularly all manifestations in the biological world, however, belong to the kinetic regime that uses equilibrium binding only as a fundamental platform for the generation of discriminatory action in subsequent rate processes. Here differences in free energies of activation rather than ground state free energies are exploited. As a rule, equilibrated systems are more amenable to molecular design than the non-equilibrium counterparts because of the consistent thermodynamic description of the former and its link to the molecular level, the chemical structures. Traditionally, this led to the quantification of selectivity as the ratio of binding constants between the guest species of interest and some competitor. Such a ratio represents a truly artificial focus on the ideal situation assuming that selectivity remains unperturbed by any other molecular species that is added to the system. In all real cases, however, thermodynamic binding selectivity is the composite outcome of complexing the target guest in the *presence* of all presumptive competitors, which may vary in a wide range with respect to chemical nature and quantity. Clearly, the ratio of binding constants enables an exact but rather hypothetical comparison of selectivity features which may fail to live up to the expectations advanced on reasonable grounds in a real application. It is all important to remember the fundamental point that selectivity in no way is an absolute quantity attributable to a certain host structure like a molecular property. Nevertheless, it is a graded measure characterizing the quality and suitability of an host design with respect to the peculiar molecular environment (which must be mentioned) and as such can report on the progress in the understanding and realization of molecular recognition.

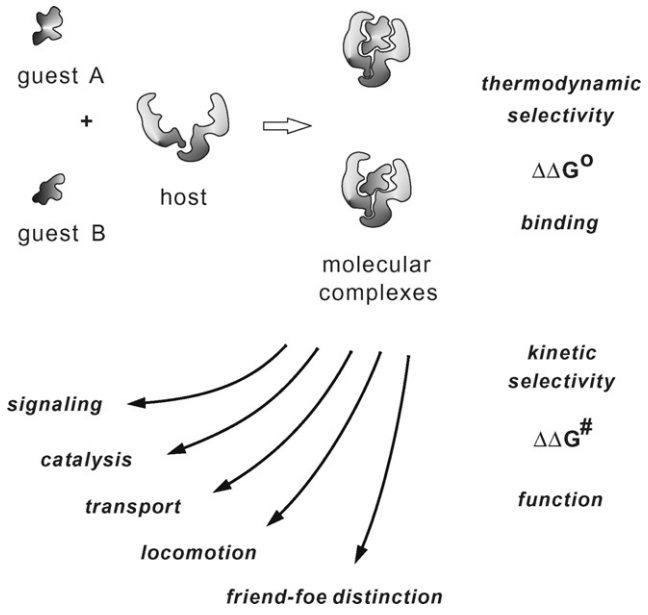


Fig. 1. Assignment of the two different domains to express selectivity.

Thermodynamic selectivity expressed as a difference in binding free energies $\Delta\Delta G$ (i.e. a ratio of binding constants) can become large only if the underlying free energies of association ΔG are even greater. Though maximizing affinity (ΔG) in principle should help thermodynamic selectivity, there is an upper limit to this strive due to practical limitations. Taking the popular vancomycin-acyl-D-ala-D-ala peptide interaction in a trimeric molecule as a prototypical example of a high affinity supramolecular complex ($K_{ass} \sim 4 \times 10^{17}\,M^{-1}$) [6] and assuming the fastest possible association of host and guest at the diffusion limit (second order rate constant in water at room temperature $\sim 10^{9}\,M^{-1}\,s^{-1}$) one can calculate a monomolecular rate constant for dissociation of the complex of $k_{off} = k_{on}/K_{ass} \cong 10^{-8}\,s^{-1}$ corresponding to a half life of about 2.5 years or roughly a generation of people for establish-

ing equilibrium. There are not many relevant processes known that depend on thermodynamic selectivity in this time regime. Fortunately, the dissociation process can be speeded up in the presence of a monotopic ligand competing in a stepwise fashion. Such kinetic bypasses are a hallmark of biological binding processes.

3. Essential concepts

It is important to realize that molecular attraction is a fundamental and unavoidable property of all matter. Whether or not the attraction between the host and guest partners yields a complex with a lifetime distinctly longer than in an average collision depends on the threshold value set by a third player on stage: the solvent. Host–guest complexation thus relates to *preferential binding* reflecting the bias of the host for the guest over the solvent [7–9]. In essence, the binding in condensed phases is a genuine exchange process replacing the solvent at the binding site by the guest molecule. As such it is distinguished from association events in the gas phase where unsolvated molecules may interact directly. The immediate comparison of the respective affinities is impossible unless extensive corrections are applied because they relate to different reactions. The participation of solvent molecules as explicit and constitutive ingredients is an essential feature of the binding process and the omission would discredit any design approach [10,11].

Solvent structure also forms the basis of another means to constrain host and guest molecules to one another even though they may lack specific attraction. The so-called solvophobic effect [12–14] is an ensemble property that shows as a tendency to minimize the surface area surrounding the solutes. Sculpturing hydrophobicity (i.e. designing solvation properties exploiting the solvophobicity of solvent water) thus is one of the most prominent and effective ways to adjust the absolute magnitude of the binding constant to a window required in some special application [15]. The clues of theses considerations for general design are obvious: binding is rather easy to achieve because the phenomenon emerges from the fundamental and ubiquitous attraction of matter, however, subject to the chemical nature of the interacting partners. Furthermore, the macroscopic observation of binding may not emanate from the subtle stickyness of the binding partners for each other. Instead, third party influences may dominate the observed outcome.

Host–guest associates are frequently presumed to possess a unique structure based on the ubiquitous observation of non-randomness in mutual binding interactions. Some moieties do interact more strongly than others leading to non-random behavior in thermal collisions. Averaging over time and the entire ensemble of molecules furnishes a distribution of species that reflects the individual preferences with respect to energy and the probability of their occurrence. This equilibrium state may be composed of a broad variety of configurationally and conformationally distinct arrangements and is subject to the molecular frameworks of the participating species including the solvent. The collection of these individual arrangements represents the structural ensemble, which resides at the heart of all design approaches. Simplifying assumptions on this terrain thus may have deep impact not only on the quantitative accuracy and reliability but also on the qualitative suitability for rationalization of the experimental outcome.

Generally, the organic chemist applies two rather crude simplifications to cut down on the overall complexity of structural ensembles to enable their ready handling: the first one ignores the structural diversity altogether and shrinks the complexity to just one basic structure. Nowadays this is frequently achieved with the help of computational molecular modeling packages by minimizing the potential energy of host and guest and finally docking the resulting structures to each other to form a topologically and geometrically well defined complex again applying energy minimization [16]. More realistic and sophisticated strategies use molecular dynamics simulations [11,17]. Positive corroboration on the validity and exactness of such calculations to the scenario in solution is claimed when the result resembles the host–guest complex structure as seen in the crystalline state. Such conclusions are deceptive on principle. Among several others, the decisive reason not to take X-ray crystal structures too seriously stems from the omission of entropy. After all, crystals are low-entropy environments and many motional degrees of freedom well excited in solution are completely silenced or replaced by lattice vibrations. The consequences of such freezing with respect to the population of different structural arrangements in any generality are totally obscure. Just one conclusion appears safe to state: even if the host–guest configuration observed in the crystal in fact were the most stable one also in solution it almost certainly would not be the only one populated owing to the much denser distribution of energy levels caused by the participation of solvent. The spreading of energy over a much larger number of microstates reflects the gain in entropy that leads to poorer structural definition of the host–guest configuration [18,19]. In synopsis, the a priori reduction of the structural diversity may compromise any design concept. On this basis interpretations can be advanced that are at variance with the physical basis of the recognition process and thus may sabotage the rationalization necessary for making progress.

On the other hand there are special advantages to the lack of structure in a host compound that relate to a greater variability of function (e.g. promiscuity [20–22], the switch of the chemical process happening in a binding site depending on the substrate bound; or moonlighting [23] the performance of different tasks at distinct locations within the host subject to the complexed guest) or to kinetic requirements as in the assembly of virus coat subunits [24] or adaptors in biological signaling networks [25]. In any case, selective binding then mandates structure generation in the associated complex leading to a correspondingly more dramatic drop in configurational entropy which must be overcompensated by the enthalpic interactions newly installed on binding. Contrary to low molecular weight host compounds macromolecular proteins can play a trick to avoid the entropic penalty [26]: binding the guest to a disordered region of the receptor yields a well defined local structure as demanded by selectivity that, however, couples to a remote moiety and triggers loosening or even complete unfolding there thus preventing a net loss in entropy. The notion transpires that natural recognition systems pay close attention to entropy factors and their

delicate balancing because of their profound influence on the global energetics. The prudent molecular architect should adopt these principles.

The second simplification introduced by host designers is the concept of anchor groups. The global molecular framework of the host is imagined to be composed of structural moieties that show some intrinsic affinity for the guest or its substructures. Generally, this division is quite arbitrary and mostly dictated by the personal preferences of the designer and by preparative necessities. The overall host–guest affinity is supposed to arise from simple additive accumulation of the individual interaction contributions. Of course, such a scheme assumes no mutual cross interactions that in principle could also be beneficial to binding if synergetic effects occur. Such positive cooperativity [27] between the various anchor groups is a very desirable feature and a strong indication of high quality design since it introduces another level in guest differentiation. For the ultimate success of the concept synergism is dispensible. In fact most examples of artificial hosts that have been constructed by modular connection of anchor groups suffer from negative cooperativity. The individual anchor groups exhibit diminished affinity when presented in the context of the modular receptor compared to the isolated moiety. This situation resembles the system of functional groups in organic chemistry at large. They invariably also show more and more unconventional physical and chemical behavior the closer their spacing and more intense their mutual communication becomes. The sensitivity to such cross-talk depends on the energy level addressed which is typically an order of magnitude lower in supramolecular interactions ($\Delta G \sim 3$–$10\,\mathrm{kJ\,mol^{-1}}$) than in the ordinary covalent chemical conversions of functional groups ($\Delta G^{\neq} \sim 50$–$130\,\mathrm{kJ\,mol^{-1}}$). The perturbation of common behavior is correspondingly much easier in the former case, a consequence that boosts the challenge of molecular design.

Another major weakness of the anchor group approach emerges from the negligence of contributions from the scaffold holding these units. They definitely participate in binding by means of desolvation or a change in configurational entropy following rigidification. Disregard of such factors could only be justified if the energy share contributed by the anchor group outmatches the contributions from the remainder by at least a power of 10. Since unspecific desolvation makes up a lion's share of the total interaction energy in more polar solvents this cannot be taken for granted visualizing the size relation, interface area and distribution between direct guest binding and non-binding fractions of the host. Though the principle of additivity that governs this approach [28] does not hold across the various types of molecular hosts known, some success has been seen within homologous series. In the absence of strong structural coupling the definition of binding increments of basic interaction types is possible [29]. As design addresses the subtleties of structure–energy interrelation these approaches are of limited help. Thus, the anchor group approach is loaded with plentiful and hard-to-prove presumptions and poorly defined restrictions. On this basis the trustful assignment of an observed effect to its true origin is rendered almost impossible.

4. The model

Models are imperfect by definition! They just reflect a fraction of preselected aspects of the total picture illuminating some portions under a narrow angle thereby producing a strongly biased projection. Of course, this projection is telling something about the original, yet an honest evaluation must appreciate the limitations in this view. This is the more difficult the simpler and easier to grasp the model is. A particularly infectious example describing host–guest binding dwells on the persuasive power of a pictorial metaphor: the lock-and-key-fit [30]. The idea of complementarity of the geometrical form giving a snug fit when the binding partners are associated like a mechanical key fits into the complementary lock of a door allowing selective entrance to the key holder is of eminent importance and has influenced the thinking of generations of chemists on supramolecular interactions. Overall the lock-and-key-model of host–guest binding is a minimalistic description of the general case addressing the binding of just two partners in an all-or-nothing two-state interaction mode based on exclusively enthalpic binding to form a singular complex. Among all host–guest binding events known such a scenario would be a great exception rather than the rule.

The caveats make a long list. Apart from the notion that host–guest association can be imagined without any enthalpic interaction between the partners (cf. solvophobic effects) it is the lack of solvent involvement that disqualifies the lock-and-key-model as a suitable means to rationalize host–guest interactions at least in the more polar solvents including water. The exclusive focus on enthalpic interactions is equivalent to the neglect of all entropic contributions to binding which is justified only in the temperature regime near zero Kelvin or in the adventitious occasion when the entropic component is nil. In reality, this situation is much less frequent than commonly anticipated and in addition also depends dramatically on the polarity of the environment. Entropic factors tend to be of greater importance in intermolecular binding in protic solvents typically required to generate anionic species as free entities in solution.

Still another aspect with bearing on the selectivity of anion binding of real host–guest systems that is commonly not addressed when using complementarity models stems from the crude assumption that the association of the anion host with its target guest is the only relevant and accountable process in solution. This implies that all other components of the system are inert and stand innocently aside. Since at least the anionic guest cannot be added to the system without an equivalent amount of counter cation, one tacitly assumes that neither the negatively charged guest nor the complex with the host will interact with the counter cation significantly, rendering all these species strong electrolytes under the solvation conditions applied. The validity of this premise is even more doubtful, if the receptor itself carries a charge. In favourable cases the interference from simultaneous ion-pairing equilibria can be included into the analytical data treatment and then may furnish a diagnostic tool to assess the complexity of the system as has been demonstrated by Gibson and coworkers [31] in the binding of organic cations to crown ethers. The involvement of simultaneous equilibria is not at all restricted to parallel reactions like ion-pairing. Also con-

secutive processes like the stepwise formation of higher order complexes may add to the complexity. Some spectacular examples in anion binding have been unravelled by Camiolo et al. [32]. Unfolding the intimacies in the sequence and strength of binding events requires a fortunate disposition of several prerequisites. Above all one needs a traceable probe acting in a suitable concentration domain (which in turn also depends on the method of analysis used) to observe the representative distribution of complex species (the speciation) and an appropriate and unchanged regime for the dynamics. Taken together these restrictions leave but a small window open to look at and investigate the situation when a guest species either acts as a competitor or a promotor of binding. The latter case features positive cooperativity [33] in which the later bound guest molecule(s) associate with stepwise higher affinity than the preceding ones. Depending on the extent of coupling between these steps, i.e. on the affinity enhancement this upward regulation may result in an on/off switch response signaling the presence or absence of the guest in a small concentration range. Generally, we should expect these sophisticated systems to mix up the effects on signaling due to the change in the concentration of the individual complex species with their certainly distinguished ability to elicit the response necessitating quite complicated models, which ultimately might not be differentiated, because of the experimental limits. The comprehension of such receptor systems can be aided by advanced level modelling adopting examples developed to explain biological phenomena (e.g. the Wyman–Monod–Changeux (WMC) or Koshland–Nemethy–Filmer (KNF) models). However, even these sophisticated models at best give a xylographic picture of higher order complexation.

5. The terms for constructing anions receptors

In order to create high-potential anion receptors by rational design four issues must be considered and correspondingly answered. The first refers to the *function* that is targeted in the construction and also includes the framing conditions. It is all important to decide at the very start on the purpose of receptor design because the goal will determine whether general affinity or rather a unique and correct structural disposition of the host-anion complex is in focus. Though these goals need not necessarily be mutually exclusive, the decision allows concentrating on the most promising route to reach the aim. For example, enhancing the affinity in a self-assembling system to meet the pertinent requirements of this process [34] would be useless if a singular (or at least dominant) interaction pattern between the building blocks cannot be guaranteed. Thus, this feature requires top priority. In turn, highly selective and strong binding of an anion to an anionophore would certainly impair the transport across a membrane, because the reversible release of the guest from a deep enthalpic well characterizing selective complexation is bound to be rather slow. In this case kinetic rather than thermodynamic considerations come into play. Distinguishing the need for structuredness or structural relaxation, respectively, is easily misguided if destruction of function is taken as advisor. The development of enzyme models which also addressed anionic substrates early on [35] provides an instructive example. Adapting the lessons learned from drug binding to their target enzymes (this process almost always aims at the functional knock out of enzymatic activity) to artificial receptors to construct abiotic enzymes falls way short of the natural activities (comparing the best examples in either series there is a gap of 10–12 orders of magnitude!). Apparently, some essential factors were not recognized or were at least left unaccounted on the transposition of the picture seen in drug/substrate binding to artificial enzyme models [10,36].

Another point of primary concern touches on the framing conditions under which the function needs to perform. In addition to requirements imposed by the goal itself (e.g. lack of bleaching in optical sensors, chemical stability in catalytic systems, lack of foaming/emulsification in extractions, etc.) the fundamental requirements with respect to solvation conditions, solubility, pH optimum or the kinetics of reversible association, etc. must be met. Experience tells that the best concepts are next to worthless if not put to work under the mandated set of conditions.

The second issue regards the *substrate* of interest, since its molecular properties finally must be recognized and distinguished from all other influences. In the case of anion sensing the first inspection must address the global structure, because in many analytes of interest the anionic moiety constitutes an important, however, not necessarily dominant part of the entire species. Take phosphate as an example. In principle, the basic interaction pattern of the phosphoryl-anionic group with a corresponding host might be very similar for inorganic orthophosphate or nucleotides, phosphatidic acids and even phosphorylated proteins. But it is evident that we are unlikely to succeed in creating an abiotic receptor capable to selectively recognize the phosphoryl anion in all these different species. If the anionic moiety is but a minor appendix of a larger substrate it may be a matter of definition and thus quite arbitrary to designate the respective receptor an anion host. In the present discussion we can restrict ourselves to the consideration of small "typical" anions, in which the net negative charge dominates the molecular properties. Here the net charge size, its density and distribution along with the overall dimensions, topology, polarizability, stereo electronics, intrinsic binding preferences, etc. must be analyzed and exploited in host design. Also in this instance naïve thinking in models might introduce an unintentional bias concerning multiply charged anions: even the mere existence of familiar anions like SO_4^{2-}, CO_3^{2-}, oxalate, etc. is connected to their molecular environment. Sulfate dianion can readily be observed in the crystalline state where it is stabilized by the lattice of countercations, or in aqueous solution there undergoing extensive hydrogen bonding. However, unsolvated sulfate has never been observed in the gas phase and is most probably thermochemically unstable, since stripping the hydrated sulfate cluster successively from its water molecules results in transprotonation ($SO_4^{2-}\cdot nH_2O \rightarrow HSO_4^{-}(n-1)\,H_2O + OH^{-}$) or Coulomb explosion [37].

In the next issue this result directs our focus to the *molecular environment*. Because anions as analytes, for electroneutrality reasons, bring in a stoichiometric number of cations these are natural competitors in any attempt aimed at supramolecular binding by anion hosts. Unspecific ion-pairing to form 1:1 com-

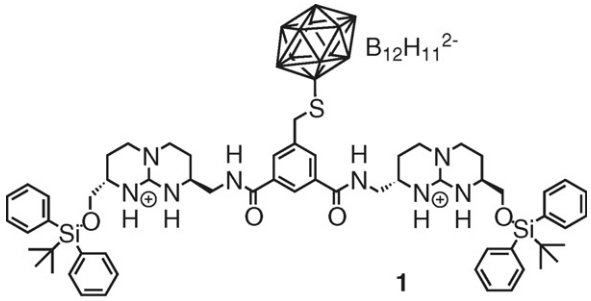
plexes or higher aggregates ($(C_n^+ A_n^-)_n$) is widespread even in water, especially with species of elevated charge. As a corollary, it is seldom justified to assume complete dissociation of salts in organic solvents of low dielectric permittivity like chloroform, dichloromethane, pyridine or the like to produce free anionic species, because fundamental Coulomb interactions will keep the concentration of free anions at minute levels. The solvent itself finally is of utmost importance as a competitor on the molecular level as it defines the threshold screen against which preferential binding must stand up. Solvent molecular size was elegantly shown to be a decisive determinant of affinity in host–guest complexation [38]. The overwhelming influence of solvation is in many cases the ultimate remedy when a less than optimal design renders an artificial host insufficiently suited to perform under the originally prospected polar solvation conditions. Switching to a solvent of minor solvating power may then serve as a final retreat and allow molecular association.

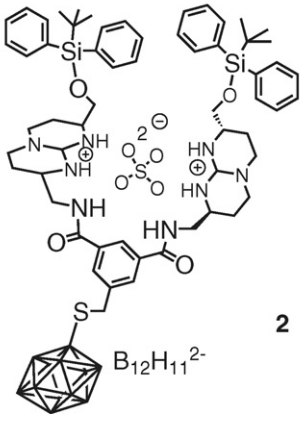
After all, the conceptually best host design would just be an intellectual exercise if the experimental realization fails. Thus, the fourth point must address the preparative *feasibility*. Generally, the synthetic expenditure is kept at minimum although a priori one might suspect a strong correlation between preparative input and the output in terms of guest selectivity. Apparently, the guidelines for design at present do not justify strong confidence into a predicted outcome making it prudent to limit the primary preparative investment. In addition to ready accessibility and modifiability which in most cases also reduces the time and cost requirement major points of concern also refer to the chemical stability and inertness under the sensing conditions. For instance, in spite of optimal synthesizability, preparative variability and sensing characteristics a potential fluorescent sensor would be soon dismissed if its chromophore is bleached or chemically harmed during measurement.

The issues relevant in the construction of anion hosts cannot be grouped in a hierarchical order as there is an intimate interplay between all of them. Successful design mandates a balanced compromise in weighting the individual influences. Advice can and should be sought from pertinent calculation (molecular modelling/dynamics), from the success and the very few examples of failure accumulated in the literature and collected in recent reviews [4,39–41], from inspiration provided by abundant biological examples and crystal structures and not the least from personal experience in the handling of host compounds and their decoration with appropriate groups to ease purification, adjustment of solubility, immobilisation and conjugation with additional moieties.

As a rule a suitable compromise of answers raised in these issues will feature a host design as an intermediate between two extremes: on one hand is the encapsulation host that aims at utmost complementarity of size and functional groups and their fixed preorganisation, resembling closely the lock-and-key picture to warrant the desired selectivity. This concept requires complete desolvation of the guest on invasion into the molecular cage provided by the host. In combination with the rigid fixation of the host structure this imposes a substantial barrier which surfaces in slow kinetics of guest equilibration and exchange. This conceptual problem may limit the utility of this strategy for sensing purposes. Furthermore, encapsulation and a rigid preorganisation of sticky binding functions require a high connectivity of the molecular framework that hampers easy access and modification of such compounds.

At the other extreme a string of anchor groups is connected covalently in branched or unbranched fashion and it is left to a folding process either prior to guest complexation or even assisted by it to arrive at a distinct three-dimensional complex structure. Many biological examples from proteins to nucleic acids to certain carbohydrates follow this strategy to bind to specific guests. Of course, lack of preorganisation leads to inferior binding given the same type and number of sticky groups in an optimally preorganized host. This price is to be paid for the ordering entropy of folding opposing binding. The enhanced flexibility and adaptability to accommodate guest structures differing from the target one also diminishes selectivity, however, the great benefits of this approach in host-design in terms of ease of preparation, modification and reuse after partial dismantlement apparently outmatches the disadvantages in selectivity generation at least for the biological examples [42].

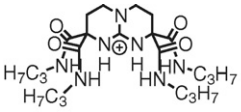




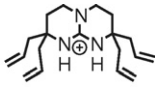
6. Addressing entropy

Supramolecular design is frequently perceived as the art to make chemical structures capable to perform defined functions. In the first instance this must address the mutual attractive and repulsive interactions of the binding partners weighted against

the influence of the molecular environment (solvent, counter ions, background electrolytes, competitive guests, etc.). As a result of such considerations the observable enthalpy arises from the opposing contributions of direct attraction between host and guest and the cost of desolvation of the common interface (the enthalpy of solvation has been found to be uniformly negative (exothermic) regardless of the solvent and the polar/unpolar nature of the interface). In principle, this balancing can furnish an endothermic outcome that taken on its own would prevent observable binding. However, in many instances including the host–guest association of anions as particularly prominent examples binding of guest to receptors takes place despite respectable heat consumption from the environment. For instance, the complexation of oxoanions to cyclic or acyclic polyammonium compounds in water almost exclusively exhibits enthalpically silent or positive responses whilst association is brought about by strongly positive entropies [43,44]. Likewise, the complexation of sulfate to a variety of bicyclic guanidinium receptors, e.g. **1** in methanol or DMSO [45] features positive enthalpies and entropies as well. Apparently, such an energetic signature is characteristic for ion-pairing processes in polar solutions, in particular, if the enthalpic attraction is of the same order of magnitude as the interaction between the solvent molecules (for a recent estimate of guanidinium sulfate association see [46]) Even in cases where a favourable enthalpy governs the energetics the entropy component most often holds a major share discouraging the naïve temptation to dismiss this fraction altogether. The participation of entropic influences as an ensemble feature in supramolecular associations in chloroform was noticed by Cram coworkers [47] 15 years ago.



3



4

More recently Jadhav and Schmidtchen [48] unfolded entropy as the true reason for the improvement of oxoanion binding by a guanidinium host that originally had been designed for enhanced enthalpic guest binding. On comparing the energetics determined by isothermal titration calorimetry (ITC) between two tetrasubstituted bicyclic guanidinium compounds **3**, **4** interacting with a variety of oxoanionic guests (Fig. 2) the affinities shown by **3** were uniformly larger than with the tetraallyl-substituted host **4** lacking the carboxamido functions. At first sight this result corroborates the idea of stronger guest binding by virtue of additional hydrogen bonding interactions that are possible with **3** only. Inspection of the underlying thermodynamic state functions, however, revealed an unexpected twist that discredited the enthalpic explanation. In every case the observed enthalpy was less attractive for the carboxamido host **3** than for the tetraallyl-substituted receptor **4**. Since the gain in entropy varied greatly among the guests despite their very similar oxoanionic structures, ordinary desolvation of the directly interacting moieties was discarded as an explanation. Obviously, other entropic rationalizations to account for the experimental result are needed, which are not related to the peculiar properties of the solvent but touch directly on the intimate host–guest relationship and hence are relevant to receptor design.

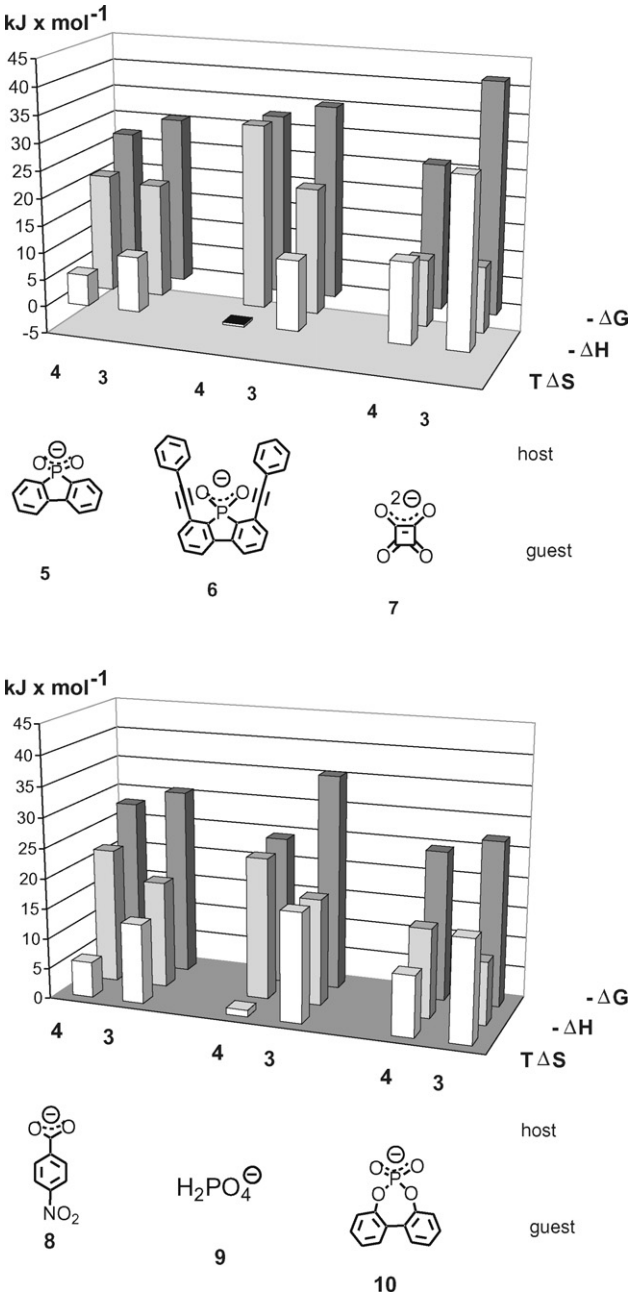


Fig. 2. Energetics of the complexation of oxoanionic guests **5**–**10** to the guanidinium hosts **3** and **4** in acetonitrile at 298 K.

Binding on entropic grounds opens a door to direct and tailor host–guest interactions that had been closed and ignored throughout most of the time of active evolution of molecular hosts presumably due to the already stated low level of "collective intuition about entropy" [49] in the supramolecular community. In essence, the consideration of entropic factors is not only a bare necessity demanded by fundamental thermodynamics, but also should provide an indispensable tool required by the implementation of a functional purpose (see above). Most current treatments of host–guest binding address the entropy issue in the context of desolvation (setting free of solvent molecules

from the interface thereby increasing the degrees of freedom and thus entropy in the system) [50]. Another, this time opposing contribution derives from the conformational restriction of the binding partners upon association. Both aspects are generally treated on a qualitative level and though they undoubtedly contribute on the balance sheet they do not cover all pertinent facets. In order to assist the general intelligibility of association entropy it is appropriate to dissect the observable output ΔS_{obs} into two component parts which are necessarily additive and refer to the solvent related change ΔS_{solv} and all alterations involving the host–guest partners $\Delta S_{intrinsic}$. This separation allows distinguishing the contributions that a priori are in a first approximation inaccessible to molecular design (ΔS_{solv}) from the fraction deserving some closer inspection with regard to the final functional goal ($\Delta S_{intrinsic}$) [51]. The latter, however, comprises again a number of additive components arising from a somewhat arbitrary dissection to aid understanding in conventional terms. The first part $\Delta S_{trans+rot}$ refers to the change in the entropy of translation and rotation of the host and guest partners on association. There is an ongoing debate on the absolute magnitude of this term [52–54], but since the dependence of entropy on molecular weight and on the moment of inertia is only weak, this term will be invariant for all practical cases of artificial receptors allowing its exclusion from design considerations. All important to molecular design, however, is the regard of the other entropic contributions addressing the freezing of internal rotations $\Delta S_{conformation}$, the generation of vibrational entropy on binding host and guest to one another $\Delta S_{vibration}$ and the occurrence of different geometrical arrangements of the binding partners $\Delta S_{configuration}$ that all may contribute appreciably to the free energy of low lying and thermally populated potential wells. The former two components:

$$\Delta S_{obs} = \Delta S_{intrinsic} + \Delta S_{solv} = \Delta S_{trans+rot} + \Delta S_{vibration} + \Delta S_{conformation} + \Delta S_{configuration} + \Delta S_{solv}$$

are closely connected since they are equally affected by the binding mechanism. If two flexible host–guest partners bind to each other loosely, internal rotations are hardly obstructed and binding vibrations will have small force constants and low bond frequencies. With respect to entropy this is a favourable situation, because the restriction of internal rotors and thus the cost in entropy on binding is small. The generation of low-frequency motion ($\nu < 1000\,cm^{-1}$) will increase entropy and augment affinity [55]. In case of tight binding caused by a deep enthalpic well internal rotations are frozen (i.e. they are in fact converted into high frequency vibrations) and the bonding motions increase in frequency and decrease in amplitude. As vibrational entropy becomes insignificant at frequencies above $1000\,cm^{-1}$ tight binding leads to a loss in entropy compared to the uncomplexed state disfavouring association. Consequently, loose and tight binding are framing options of the entropy component that are open to the creativity of the molecular designer to follow her/his goal. If pure binding affinity is at the top of the list of wishes as necessary for sequestration or in the extraction of anions the maximization of association entropy avoiding enthalpically tight binding offers an appropriate handle. Conversely, if the desired function vitally depends on a unique structure of the host–guest complex as is true in regioselective/stereoselective binding and catalysis or in self-assembly, respectively, the association entropy should be minimized. Of course, the absolute magnitude and even the sign of the experimentally determined entropy ΔS_{obs} cannot serve as a guide in this respect. On one hand $\Delta S_{vibration}$ and $\Delta S_{conformation}$ are just two in a number of component parts and they are likely not the dominant ones. Furthermore, another component relating to the various and quite distinct geometrical configurations of host and guest $\Delta S_{configuration}$ may interfere as well. It is conceivable that the host–guest pair will find several if not many potential wells characterized by distinct distances and orientations of the partners yet possessing similar energy subject to their size and flexibility. Even if tight binding occurs in each of these wells, the macroscopic structural definition of the interconverting ensemble of populated wells is compromised. Such ambiguity can be seen with highly restricted artificial hosts like the cyclodextrins binding certain drugs of elongated shape. A recent computational analysis [56] reveals not only different threading modes (head-on versus tail-on) of the stick-like guests into the hollow core of α, β or γ-cyclodextrins but also a change in this threading mode in a few of the 20 configurations of lowest energy of the naproxene-β-cyclodextrin complex which all are populated. Less rigid host–guest pairs certainly are likely to have much broader distributions of geometrical arrangements. With the ordinary tools of structural analysis (NMR!) the structural diversity goes unnoticed owing to rapid averaging. A good pictorial expression of the variety of host–guest binding modes is provided by the overlay of solid state structures of phosphate diester anion binding to guanidinium anchor groups as occurring on winding DNA around the arginine-rich histones in nucleosomes [57] (Fig. 3). Compared to simple oxoanion salts of guanidines which almost invariably feature a bidentated in plane structural motif of this host–guest pair in the crystal the inhomogeneity of the protein environment mimicking the fluctuation in the solution state leads to a diversity of configurations that covers almost an entire hemisphere.

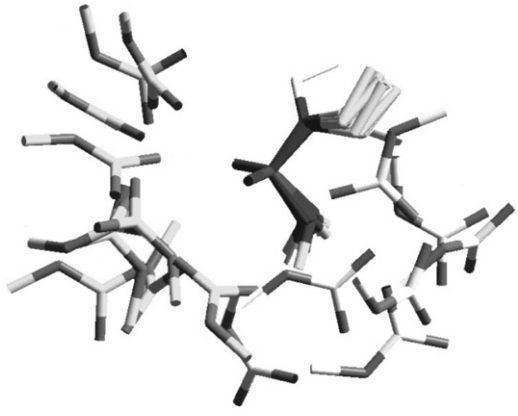


Fig. 3. Overlay of X-ray crystal structures of guanidinium groups interacting with the phosphodiester groups (center) of DNA when wound around the arginine-rich histone protein complex in nucleosomes [57]. Reproduced with permission of Wiley Periodicals.

In order to constrain the variety of modes the guanidinium moiety was embedded into a bicyclic framework and further decorated with substituents in the α, α′-positions to limit the accessibility to the binding site. Taking benzoate as an oxoanionic probe a massive influence of the guanidinium counter anion on binding affinities was noticed [58]. In the series chloride, bromide, iodide, hexafluorophosphate, tetrafluoroborate the affinity increased by more than 10-fold in this sequence indicating the participation of ion-pairing following the hydrogen bond acceptor capacity of the counter anion. The effect of the lining of the binding site by various more or less bulky hydrocarbon substituents (**11**, **13**–**15**) was only moderate and was accommodated by desolvation arguments. Presumably due to the leanness and distance of the bound benzoate from the substituents attached to the host no influence on the variety of binding modes could be deduced from the energetics [58]. The situation changed dramatically when binding site accessibility was limited also at the guest anion. Calorimetry of the series of guanidinium hosts **11**–**15** with three phosphinate guests **16**–**18** of increasing steric congestion allowed pinpointing the effect of configurational entropy. While the entropy production on association with the parent phosphinate **16** was uniform and strongly positive assisting the exothermic binding that was seen in all cases, the attachment of hydrocarbon side arms to the flanking positions diminished the entropy output and even gave negative values in few cases. Such lowering of the association entropy with **17** and **18** cannot emerge from a general desolvation effect since both enthalpy and entropy of solvation scale with the size of the interface area in the assembled host–guest complex. Viewing the dimensions the interface must undoubtedly be larger in the case of the concave guests leading to the expectation that the desolvation should give more positive entropy relative to the parent phosphinate **16**. From the observation of more negative entropies one must suspect a different origin. The change in

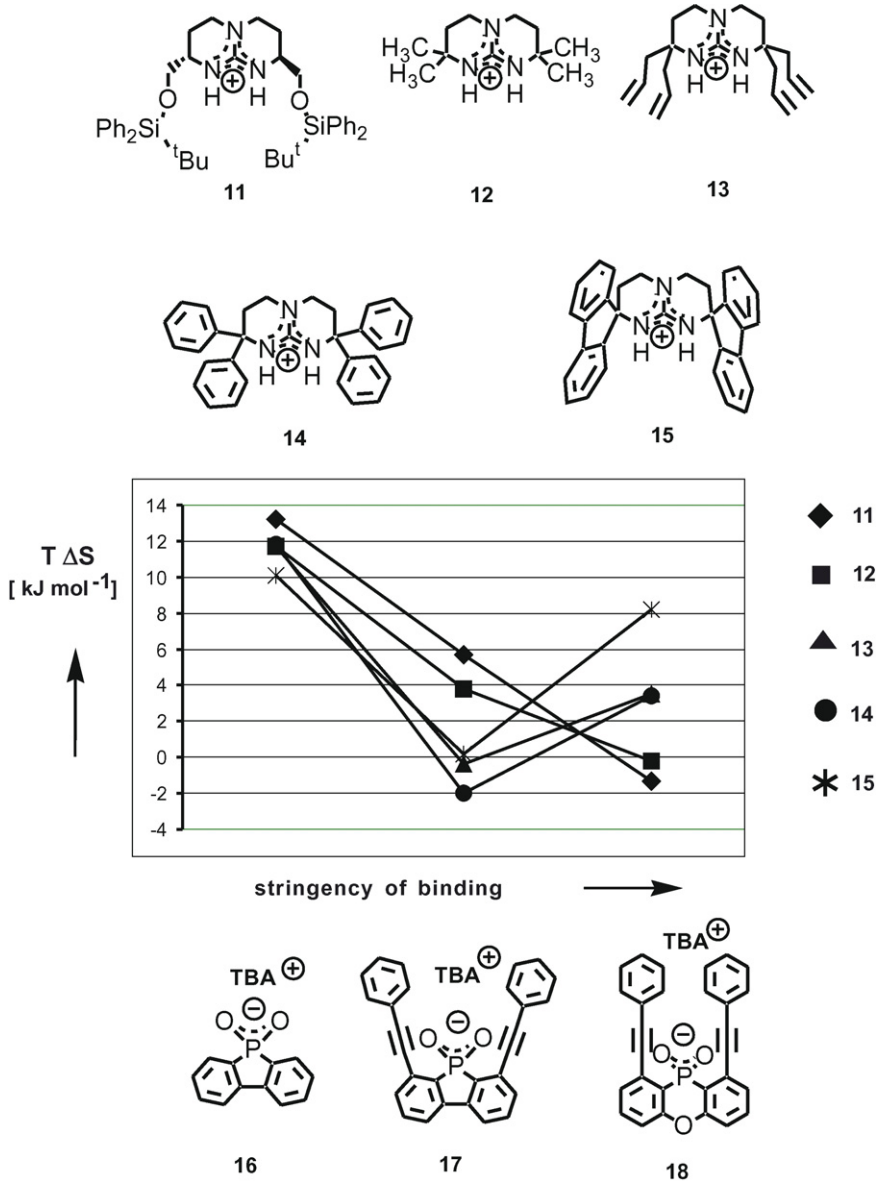


Fig. 4. Trend analysis of the observed entropy in the association of bicyclic guanidines **11**–**15** with restricted phosphinates **16**–**18** in acetonitrile at 298 K.

$\Delta S_{trans+rot}$ and $\Delta S_{conformation}$ is nearly identical throughout the series of phosphinates due to the almost identical size and rigidity of the guests. The two remaining contributions $\Delta S_{configuration}$ and $\Delta S_{vibration}$ to the intrinsic entropy $\Delta S_{intrinsic}$ are expected to depend on the mutual fit of the interacting partners. A snug fit would represent a unique binding mode corresponding to the lock-and-key picture that also should give rise to high frequencies in intermolecular bonding. The respective entropy components must then be at a minimum on both counts. All other situations should give more positive returns in entropy. Such a scenario was experimentally verified and yielded the plot depicted in Fig. 4 [59]. Within the series of guanidinium hosts **11**–**15** and phosphinate guests **16**–**18** the analysis of the entropy-based contribution ($T\,\Delta S$) to the free energy reveals a distinct minimum for host–guest combinations that balance the stringency of interaction. Amazingly, these pairs also feature an almost identical entropy output supporting the notion that they represent a singular state of the complex. Thus, only these combinations, however, not the remaining ones resemble the lock-and-key picture. Structural uniqueness is also backed by the highest exothermicities found indicating best "stickyness" and the lowest tendency to engage in the formation of higher order complexes.

This example demonstrates that the inspection of association entropies can provide a clue to rationally select the most suitable host–guest combination for a certain function. In the concrete case the utility of the guanidinium-phosphinate motif as a supramolecular tecton to erect larger assemblies was probed yielding a satisfactory answer by the identification of the structurally best defined pairs.

As the observable entropy is considered a composite of several influences, prudent decisions cannot be advanced on a singular case. Instead, trend analyses on ensembles of hosts and guests differing systematically within the series are required. Of course, such an approach multiplies the experimental expenditure, but opens the perspective to arrive at a founded conclusion on the structuredness of host–guest interactions. The result could be all important to successful design.

7. Conclusion

The impact of entropy as a constitutive and ubiquitous part of supramolecular design has long been neglected and many failures in the past to implement supramolecular thinking into useful applications in retrospect have been hampered by this omission. There is cause for optimism though! One promising route across the computational chemistry terrain leads to the development of force fields that are calibrated in overall thermodynamic state functions to dim the structural bias of present day modelling [11,60]. Undoubtedly, this will promote the entropic predictability of host–guest systems, which is rather vague at present [61,62]. Another hopeful prospect emerges from the availability of sound experimental entropies deriving from the advent and wide-spread use of convenient isothermal titration calorimeters. Despite the subtleties of entropy component deconvolution there is NO sign to resign from design. Just another dimension is opened that requires the serious appreciation of structural diversity and surely adds to the complexity of the challenge of design. To cope with this situation necessitates to re-think popular models. The master said it before [63]:

> "Your model should be as simple as possible – but not simpler!"

Acknowledgement

The continuous support of our work by Hans-Fischer-Gesellschaft, Munich, and Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References

[1] In a recent article (Angew. Chem. Int. Ed. 45 (2006) 3406) M. Jansen and J.C. Schön question the possibility of design (taken in a geometrical sense) in the synthesis of molecular objects (e.g. a molecular host). The main argument rests on the finite number of equilibrium arrangements of a set of atoms/molecules constituting a chemical system. This restriction predisposes any possible result in construction and reduces "design" to a selection process from a limited variety of options. In our concrete subject we deal with the translation of supramolecular atomistic relations into macroscopic properties on a molar level comprising $10^{26,000,000,000,000,000,000}$ microstates even at very low temperature ($T < 4$ K, K.S. Pitzer, *Thermodynamics*, 3rd ed., McGraw-Hill, New York, 1995, p. 67; cf. E.I. Kozliak, F. Lambert, Chem. Educator 10 (2005) 24). The fraction among these microstates that form equilibrium arrangements of all atoms and are indeed populated at room temperature may be substantially smaller. Even though, their number will still be immense preventing any testing of the individual cases. We believe, thus, that there is room enough for knowledge, intuition, experience and sensible feeling in the construction of molecular receptors to justify the term "design" as it relates to an act of creativity.

[2] For pertinent summaries see:
(a) J.L. Sessler, P.A. Gale, W.-S. Cho, Anion Receptor Chemistry, RCS Publishing, Cambridge, 2006;
(b) A. Bianchi, K. Bowman-James, E. García-Espana (Eds.), Supramolecular Chemistry of Anions, Wiley–VCH, New York, 1997;
(c) S. Kubik, C. Reyheller, S. Stuewe, J. Inclus. Macrocycl. Chem. 52 (2005) 137;
(d) P.D. Beer, P.A. Gale, Angew. Chem. Int. Ed. 40 (2001) 486;
(e) F.P. Schmidtchen, M. Berger, Chem. Rev. 97 (1997) 1609.

[3] F.P. Schmidtchen, Angew. Chem. Int. Ed. Engl. 16 (1977) 720.

[4] (a) F.P. Schmidtchen, Top. Curr. Chem. 255 (2005) 1;
(b) A. Bianchi, E. García-Espana, Thermodynamics of anion complexation, in: A. Bianchi, K. Bowman-James, E. García-Espana (Eds.), Supramolecular Chemistry of Anions, Wiley–VCH, New York, 1997, p. 217.

[5] F.P. Schmidtchen, Thermodynamic and kinetic selectivity, in: J. Atwood (Ed.), Encyclopedia of Supramolecular Chemistry, Marcel Dekker, New York, 2004, p. 1225.

[6] J. Rao, J. Lahiri, R.M. Weis, G.M. Whitesides, J. Am. Chem. Soc. 122 (2000) 2698.

[7] S.N. Timasheff, Biophys. Chem. 101/102 (2002) 99.

[8] J.A. Schellman, Biophys. Chem. 96 (2002) 91.

[9] (a) A. Ben-Naim, Biophys. Chem. 101/102 (2002) 309;
(b) A. Ben-Naim, Biophys. Chem. 105 (2003) 183.

[10] H. Gohlke, G. Klebe, Angew. Chem. Int. Ed. 41 (2002) 2644.

[11] W.F. van Gunsteren, D. Bakowies, R. Baron, I. Chandrsekhar, M. Christen, X. Daura, P. Gee, D.P. Geerke, A. Glättli, P.H. Hünenberger, M.A. Kastenholz, C. Oostenbrink, M. Schenk, D. Trzesniak, N.F.A. van der Vegt, H.B. Yu, Angew. Chem. Int. Ed. 45 (2006) 4064.

[12] L.R. Pratt, Ann. Rev. Phys. Chem. 53 (2002) 409.

[13] S. Moelbert, P. De Los Rios, J. Chem. Phys. 119 (2003) 7988.

[14] S. Yasuda, I. Suzuki, K.-I. Shinohara, H. Shigekawa, Phys. Rev. Lett. 96 (2006) 228303.

[15] For an early example of making use of solvophobic binding in artificial hosts see: D.J. Cram, H.-J. Choi, J.A. Bryant, C.B. Knobler, J. Am. Chem. Soc. 114 (1992) 7748.
[16] cf.K.B. Lipkowitz, J. Chem. Educ. 72 (1995) 1070.
[17] S.A. Adcock, J.A. McCammon, Chem. Rev. 106 (2006) 1589.
[18] For illustrative examples see:
(a) A. Glättli, W.F. van Gunsteren, Angew. Chem. Int. Ed. 43 (2004) 6312;
(b) R. Barron, D. Bakowies, W.F. van Gunsteren, J. Pept. Sci. 11 (2005) 74;
(c) D. Trzesniak, A. Glättli, B. Jaun, W.F. van Gunsteren, J. Am. Chem. Soc. 127 (2005) 14320.
[19] C.-E. Chang, M.K. Gilson, J. Am. Chem. Soc. 126 (2004) 13156.
[20] V.N. Uversky, C.J. Oldfield, A.K. Dunker, J. Molec. Recognit. 18 (2005) 343.
[21] A.L. Hopkins, J.S. Mason, J.P. Overngton, Curr. Opin. Struct. Biol. 16 (2006) 127.
[22] A.L. Fink, Curr. Opin. Struct. Biol. 15 (2005) 35.
[23] S.D. Copley, Curr. Opin. Chem. Biol. 7 (2003) 265.
[24] A. McPherson, BioEssays 27 (2005) 447.
[25] A. Csiszár, BioEssays 28 (2006) 465.
[26] (a) C.S. Verma, S. Fischer, Biophys. Chem. 115 (2005) 295;
(b) D.H. Williams, M. Zhou, E. Stephens, J. Mol. Biol. 355 (2006) 760.
[27] (a) D.H. Williams, C.T. Calderone, D.P. O'Brien, R. Zerella, Chem. Commun. (2002) 1266;
(b) D.H. Williams, D.P. O'Brien, A.M. Sandercock, E. Stephens, J. Mol. Biol. 340 (2004) 373;
(c) D.H. Williams, N.L. Davies, R. Zeralla, B. Bardsley, J. Am. Chem. Soc. 126 (2004) 2042.
[28] K.A. Dill, J. Biol. Chem. 272 (1997) 01.
[29] A. De Robertis, C. De Stefano, O. Giuffre, S. Sammartano, Ann. Chim. 89 (1999) 23.
[30] E. Fischer, Chem. Ber. 27 (1894) 2985.
[31] (a) J.W. Jones, H.W. Gibson, J. Am. Chem. Soc. 125 (2003) 7001;
(b) J.W. Jones, F. Huang, W.S. Bryant, H.W. Gibson, J. Am. Chem. Soc. 45 (2004) 5961;
(c) F. Huang, J.W. Jones, C. Slebodnik, H.W. Gibson, J. Am. Chem. Soc. 125 (2003) 14458.
[32] S. Camiolo, P.A. Gale, M.B. Hursthouse, M.E. Light, Org. Biomol. Chem. 1 (2003) 741.
[33] G. Ercolani, Org. Lett. 7 (2005) 803.
[34] (a) G. Ercolani, J. Phys. Chem. B 107 (2003) 5052;
(b) G. Ercolani, J. Am. Chem. Soc. 125 (2003) 16097.
[35] (a) F.P. Schmidtchen, Angew. Chem. Int. Ed. Engl. 20 (1981) 466;
(b) F.P. Schmidtchen, Chem. Ber. 117 (1984) 1287;
(c) F.P. Schmidtchen, J. Mol. Catal. 37 (1986) 141;
(d) F.P. Schmidtchen, Top. Curr. Chem. 132 (1986) 101.
[36] D.H. Williams, E. Stephens, D.P. O'Brien, M. Zhou, Angew. Chem. Int. Ed. 43 (2004) 6596.
[37] (a) A.T. Blades, P. Kebarle, Can. J. Phys. Chem. A 109 (2005) 8293;
(b) D. Schroeder, Angew. Chem. Int. Ed. 43 (2004) 1329.
[38] K.T. Chapman, W.C. Still, J. Am. Chem. Soc. 111 (1989) 3075.
[39] K. Bowman-James, Acc. Chem. Res. 38 (2005) 671.
[40] S. Kubik, Cyclopeptides as macrocyclic host molecules for charged guests, in: C. Schmuck, H. Wennemers (Eds.), Highlights in Bioorganic Chemistry, Wiley–VCH, Weinheim, 2005, p. 124.
[41] C. Suksai, T. Tuntulani, Top. Curr. Chem. 255 (2005) 163.
[42] An increase in order does not necessarily involve a diminution of entropy, see:
(a) H.N.W. Lekkerkerker, A. Stroobants, Nature 393 (1998) 305;
(b) M. Adams, Z. Dogic, S.L. Keller, S. Fraden, Nature 393 (1998) 349.
[43] H.J. Schneider, V. Ruediger, O.A. Raevsky, J. Org. Chem. 58 (1993) 3648.
[44] C. Bazzicalupi, A. Bencini, A. Bianchi, M. Cecchi, B. Escuder, V. Fusi, E. Garcia-espana, C. Giorgi, S.V. Luis, G. Maccagni, V. Marcelino, P. Paoletti, B. Valtancoli, J. Am. Chem. Soc. 121 (1999) 6807.
[45] (a) M. Berger, F.P. Schmidtchen, Angew. Chem. Int. Ed. 37 (1998) 2694;
(b) M. Berger, F.P. Schmidtchen, J. Am. Chem. Soc. 118 (1996) 8947;
(c) M. Berger, F.P. Schmidtchen, J. Am. Chem. Soc. 121 (1999) 9986.
[46] P.E. Mason, C.E. Dempsey, G.W. Neilson, J.W. Brady, J. Phys. Chem. B 109 (2005) 24185.
[47] J.A. Bryant, J.L. Ericson, D.J. Cram, J. Am. Chem. Soc. 112 (1990) 1255.
[48] V.D. Jadhav, F.P. Schmidtchen, Org. Lett. 11 (2005) 331.
[49] G. Ercolani, J. Org. Chem. 64 (1999) 3350.
[50] (a) S.L. Tobey, E.V. Anslyn, J. Am. Chem. Soc. 125 (2003) 14807;
(b) S.L. Wiskur, J.J. Lavigne, A. Metzger, S.L. Tobey, V. Lynch, E.V. Anslyn, Chem. Eur. J. 10 (2004) 3792;
(c) S.L. Tobey, E.V. Anslyn, J. Am. Chem. Soc. 125 (2003) 10963.
[51] For a lucid discussion of the dissection of thermodynamic state functions see:
(a) D.H. Williams, M.S. Westwell, Chem. Soc. Rev. 27 (1998) 57;
(b) D.H. Williams, E. Stephens, M. Zhou, Chem. Commun. (1973–1976), and earlier work of these authors.
[52] B. Lu, C.F. Wong, Biopolymers 79 (2005) 277.
[53] C.W. Murray, M.L. Verdonk, J Comp. Aid. Mol. Design 16 (2002) 741.
[54] B.Y. Yu, P.L. Privalov, R.S. Hodges, Biophys. J. 81 (2001) 1632.
[55] J.D. Dunitz, Chem. Biol. 2 (1995) 709.
[56] W. Chen, C.-E. Chang, M.K. Gilson, Biophys. J. 87 (2004) 3035.
[57] M. García-Pérez, M. Pinto, J.A. Subirana, Biopolymers 69 (2003) 432.
[58] M. Haj-Zaroubi, N.W. Mitzel, F.P. Schmidtchen, Angew. Chem. Int. Ed. 41 (2002) 104.
[59] M. Haj-Zaroubi, F.P. Schmidtchen, Chem. Phys. Chem 6 (2005) 1181.
[60] M. Christen, P.H. Hunenberger, D. Bakowies, R. Baron, R. Burgi, D.P. Geerke, T.N. Heinz, M.A. Kastenholz, V. Krautler, C. Oostenbrink, C. Peter, D. Trzesniak, W.F. van Gunsteren, J. Comp. Chem. 26 (2005) 1719.
[61] S.-T.D. Hsu, C. Peter, W.F. van Gunsteren, A.M.J.J. Bonvin, Biophys. J. 88 (2005) 15.
[62] K.L. Mardis, J. Phys. Chem. B 110 (2006) 971.
[63] A. Einstein, cited after: http://www.janko.at/Zitate/Autoren/Einstein.htm.