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Jurriaan Huskens, Leonard J. Prins,
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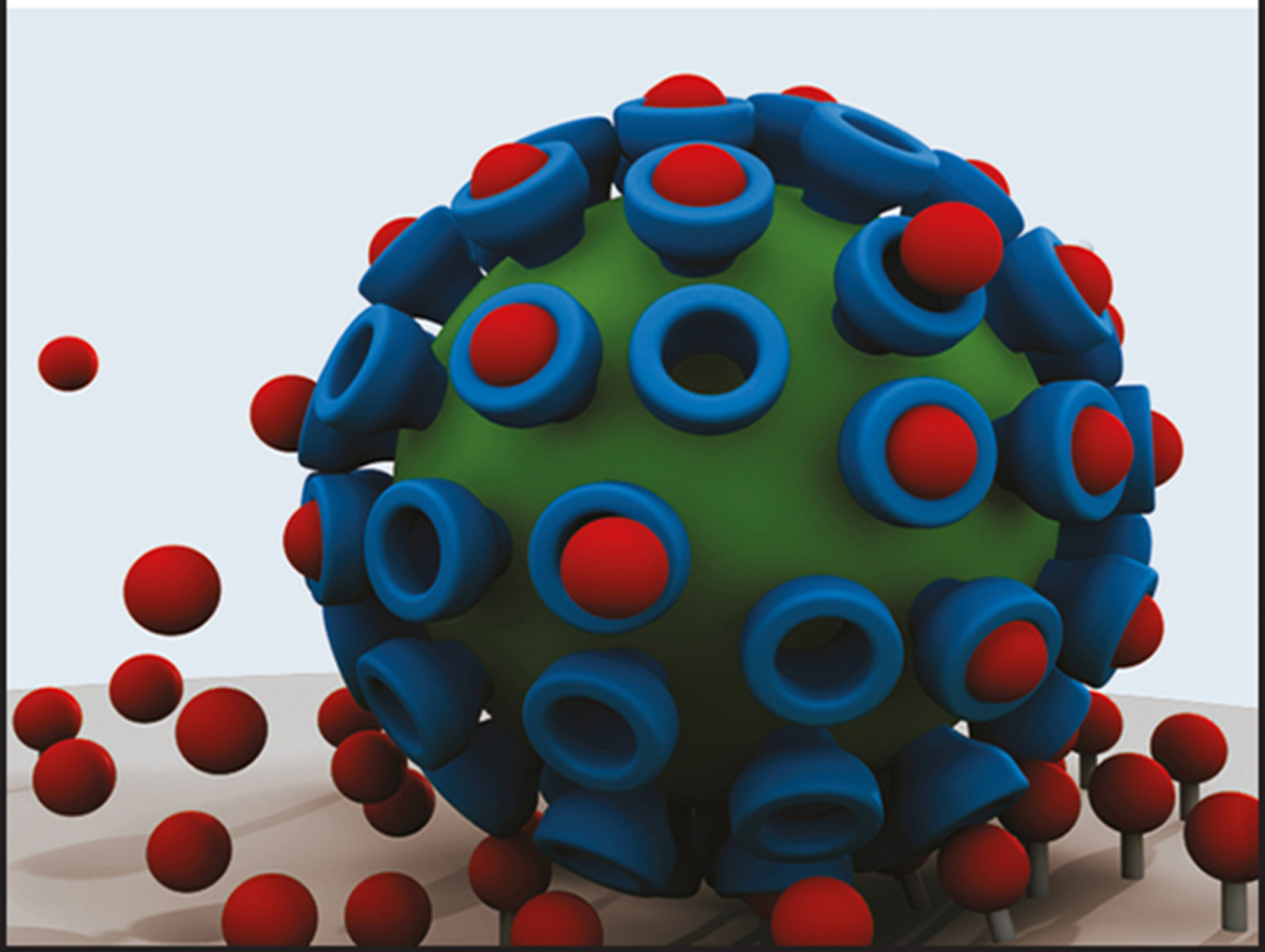
Multivalency

Concepts, Research & Applications



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Edited by

Jurriaan Huskens
University of Twente
Enschede, the Netherlands

Leonard J. Prins
University of Padova
Italy

Rainer Haag
Freie Universität Berlin
Germany

Bart Jan Ravoo
Westfälische Wilhelms-Universität Münster
Germany

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Contents

List of Contributors

Sumati Bhatia
Institute of Chemistry and Biochemistry
Freie Universität Berlin
Germany

Luc Brunsveld
Department of Biomedical Engineering
Laboratory of Chemical Biology and Institute of Complex Molecular Systems
Eindhoven University of Technology
The Netherlands

Maria A. Cardona
Department of Chemical Sciences
University of Padova
Italy

Alessandro Casnati
Department of Chemistry
Life Sciences and Environmental Sustainability
Università di Parma
Italy

Emanuela Cavatorta
MESA+ Institute for Nanotechnology & MIRA Institute for Biomedical Technology and Technical Medicine
University of Twente
Enschede
The Netherlands

Tine Curk
Department of Chemistry
University of Cambridge
UK

Jens Dernedde
Institute of Laboratory Medicine, Clinical Chemistry, and Pathobiochemistry
Charité–Universitätsmedizin Berlin
Germany

Jure Dobnikar
Institute of Physics & School of Physical Sciences
Chinese Academy of Sciences
Beijing
China

Daan Frenkel
Department of Chemistry
University of Cambridge
UK

Akash Gupta
Department of Chemistry
University of Massachusetts, Amherst
USA

Rainer Haag
Institute of Chemistry and Biochemistry
Freie Universität Berlin
Germany

Akira Harada
Graduate School of Science
Osaka University
Japan

Akihito Hashidzume
Graduate School of Science
Osaka University
Japan

Zehuan Huang
Department of Chemistry
Tsinghua University
Beijing
China

Jurriaan Huskens
MESA+ Institute for Nanotechnology
University of Twente
Enschede
The Netherlands

Pascal Jonkheijm
MESA+ Institute for Nanotechnology
& MIRA Institute for Biomedical
Technology and Technical Medicine
University of Twente
Enschede
The Netherlands

Ulrike Kauscher
Organic Chemistry Institute
Westfälische Wilhelms-Universität
Münster
Germany

Carlos M. León Prieto
Department of Chemical Sciences
University of Padova
Italy

Bernd Lepenies
Immunology Unit & Research Center for
Emerging Infections and Zoonoses (RIZ)
University of Veterinary
Medicine Hannover
Germany

João T. Monteiro
Immunology Unit & Research Center for
Emerging Infections and Zoonoses (RIZ)
University of Veterinary
Medicine Hannover
Germany

Roland J. Pieters
Department of Chemical Biology & Drug
Discovery
Utrecht Institute for Pharmaceutical
Sciences
Utrecht University
The Netherlands

Leonard J. Prins
Department of Chemical Sciences
University of Padova
Italy

Bart Jan Ravoo
Organic Chemistry Institute
Westfälische Wilhelms-Universität
Münster
Germany

Moumita Ray
Department of Chemistry
University of Massachusetts, Amherst
USA

Vincent M. Rotello
Department of Chemistry
University of Massachusetts, Amherst
USA

Francesco Sansone
Department of Chemistry
Life Sciences and Environmental
Sustainability
Università di Parma
Italy

Hans-Jörg Schneider
FR Organische Chemie
Universität des Saarlandes
Saarbrücken
Germany

Paolo Scrimin
Department of Chemical Sciences
University of Padova
Italy

Marjon Stel
Department of Chemical Biology & Drug Discovery
Utrecht Institute for Pharmaceutical Sciences
Utrecht University
The Netherlands

Jens Voskuhl
Institute of Organic Chemistry
University of Duisburg-Essen
Germany

Xi Zhang
Department of Chemistry
Tsinghua University
Beijing
China

Benjamin Ziem
Institute of Chemistry and Biochemistry
Freie Universität Berlin
Germany

Foreword

Scientific challenges come and go; only a few of them remain for a long time. Multivalency is one of those research topics that has been prominent for many years, as this intriguing phenomenon is of profound importance in many biological processes as well as very difficult to understand and mimic. Personally, I became intrigued by the challenge of multivalency when our group entered the field of dendrimers in 1990. The controlled number of end groups – 4, 8, 16, 32, and 64 amines of the polypropylene imines – opened many opportunities for us to explore the controlled use of multiple interactions. However, our ideas were more simple than our experiments in making full use of the potential of multivalency; many of them remained in the realm of dreaming. The broad potential of multivalency as well as its complex mode of action was beautifully illustrated by George Whitesides and coworkers [1] in the seminal *Angewandte Chemie* review paper in 1998. Their review initiated a world-wide search for synthetic mimics of these highly effective natural systems, a search that turned out to be long lasting.

Nature uses both similar interactions (homovalency) and different interactions (heterovalency) to control selectivity and specificity, even leading to ultra-sensitivity. Beautiful examples are found in substrate–cell interactions and immunology. Ever since this elegant mechanism and its importance in biological systems has been recognized, chemists have been intrigued to fully understand the enhancement factors obtained in binding multiple weak interactions through multivalency. Artificial systems are designed, synthesized, and studied, while a number of applications are proposed. Multivalent medication can have lower toxicity while simultaneously having higher medical efficacy.

Although the knowledge on the modus operandi of these systems has increased significantly in time and the systems synthesized have become more active, the full potential of the proposed applications remains. Hence, a number of challenging questions need to be answered before the potential of this intriguing concept can be explored. How to design the ideal structure to arrive at the theoretical maximum avidity and how to obtain scaling with valency are just a few of these intriguing questions. Theoretical and experimental studies of multivalent systems have revealed several design parameters that are critical in obtaining effective multivalent constructs. Next to the binding affinity, linker flexibility plays an important role, as rigid linkers require extremely precise ligand positioning to obtain high binding affinities and selectivity, while flexible linkers offer more freedom in molecular design at the cost of lower affinity and selectivity. Furthermore, additional competing equilibria can be used to enhance binding

selectivity or to steer an assembly towards a preferred state. However, the complexity of all these effects and their interference makes the field one of the most challenging areas in the molecular sciences.

Therefore, it is great to see that four outstanding scientists have edited a book on the intriguing topic of multivalent interactions. It is a book full of excellent chapters written by the most active experts in the field, covering all aspects of multivalent interactions with special emphasis on theory, synthesis, surfaces, chemical biology, and supramolecular chemistry. I am convinced that this book will be a great asset for all active in this intriguing field of science.

Eindhoven, May 2017 *E.W. Meijer*

Reference

1 Mammen, M., Choi, S.-K., Whitesides, G. M. Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794.

Preface

Multivalent interactions play a role in molecular and biomolecular systems in which molecules interact by multiple noncovalent bonds. Studying and describing these interactions in a quantitative manner constitute therefore an important way to obtain insight into the functional behavior of the biological and chemical systems in which they are involved. Over the past decades, the research of multivalent interactions has greatly expanded. This growth fits in the overall trends observed in the natural sciences which encompass the merging and overlapping of disciplines, like the biology and chemistry involved here. It also aligns with the emphasis on the study of complex systems, and the development of systems biology and systems chemistry, for example. Therefore, we have observed the need for a book that brings together fundamental aspects of multivalent interactions and relevant current examples of biological as well as chemical multivalent systems.

The disciplines of chemistry and biology are strongly represented in this area of science because they exert a mutual influence on both the understanding of fundamental aspects of multivalency as well as the development of practical research tools and applications. In biology, multivalent interactions play an eminent role in the immune system, but at the same time also describe the interactions between a virus and the host cell which the virus tries to infect. Tools from chemistry and nanotechnology are being developed that assist in studying such complex biological systems, for example, by synthesizing model cell membranes in which the interactions can be studied in a more controllable fashion. Likewise, probe techniques allow quantification of interactions at the single molecule level in individual cells. Conversely, the increase in understanding of the biomolecular interactions in living systems sparks the generation of new types of drugs and inhibitors that can make smart use of the multivalent character to improve both selectivity and activity.

A quantitative understanding of multivalent interactions is essential to promote progress in the field that deals with multivalent systems. Both experimental techniques as well as modeling can be used to stimulate this depth of understanding. Therefore, we decided that chapters with a strong educational character should be an essential part of this book. We present a section (Part I) of four chapters that serve to guide new researchers as well as more experienced researchers in their efforts to contribute to this lively area. These chapters provide a background in thermodynamics, data modeling and the description of multivalent equilibrium systems, numerical modeling of multivalent systems and superselectivity, and an introduction to multivalent biological systems. These chapters build on, and for some aspects briefly review, knowledge that most readers

with a background in chemistry or biology will have encountered in their regular academic education, but from there quickly integrate this knowledge into the description of multivalent systems.

Another explicit aim of the book is to expose the active nature of the research on multivalent systems. This is achieved in the two other sections of the book (Parts II and III), dealing with chemical and biological examples of multivalency, respectively. In the chemistry oriented chapters, timely topics such as the host–guest interactions of cyclodextrins and cucurbiturils are covered, as well as soft matter systems, such as vesicles, polymers, and nanoparticles. Not only equilibrium thermodynamics is shown, but also systems in which multivalent interactions control catalysis. In the more biological section, several biological interactions are put forward, such as protein–protein and lectin–glycan interactions. The strong connection between chemistry and biology in this area is emphasized by the examples that describe cell targeting by molecules and nanoparticles, as well as receptor inhibition by multivalent inhibitors.

We hope that this book will serve a need, for new and experienced researchers alike, both for those requiring a deeper understanding as well as those that try to get an overview of existing activities in the field. We thank all contributing authors for their efforts in summarizing and describing their research and that of others, as their joint work makes this book so much more than the individual chapters alone. We also express our gratitude to the Wiley staff for smoothing the pathway for the book that lies before you.

September 2017

Jurriaan Huskens, Leonard J. Prins, Rainer Haag, and Bart Jan Ravoo

Part I

General Introduction to Multivalent Interactions

1

Additivity of Energy Contributions in Multivalent Complexes

Hans-Jörg Schneider

FR Organische Chemie, Universität des Saarlandes, 66123 Saarbrücken, Germany

1.1 Introduction

Additivity of individual binding contributions is the very basis of multivalency. In classical coordination chemistry such simultaneous actions are described as the chelate effect. They offer almost unlimited ways to enhance the affinity [1,2,3,4,5,6], and therefore within certain limitations also the selectivity [7] of synthetic and natural complexes. Although additivity is often implied in experimental and theoretical approaches it is subject to many limitations which will be also discussed in the present chapter.

1.2 Additivity of Single Interactions – Examples

If only one kind of interaction is present in a complex one can expect a simple linear correlation between the number n of the individual interaction free energies $\Delta\Delta G_i$ and the total ΔG_t (Equation 1.1), as illustrated in Figure 1.1 for salt bridges [8]. Even though the organic ion pair complexes are based on cations and anions of very different size and polarizability one observes essentially additive salt bridges; the slope of the correlation indicates an average of $\Delta\Delta G = (5 \pm 1)$ kJ/mol per salt bridge. The value of (5 ± 1) kJ/mol is observed in usual buffer solution, but varies as expected from the Debye–Hückel equation with the ionic strength of the solution [9]. Scheme 1.1 shows a corresponding value of $K \approx 10\,M^{-1}$ per salt bridge for typical complexes where the affinity depends as expected on the degree of protonation [7].

$$\Delta G_t = n \cdot \Delta\Delta G_i \tag{1.1}$$

The additivity depicted in Figure 1.1 and Scheme 1.1 for salt bridges is in line with the Bjerrum equation, which describes ion pair association as a function of the ion charges z_A and z_B; Figure 1.2 shows for over 200 ion pairs a linear dependence of $\log K$ vs. $z_A z_B$ [3]. For inorganic salts one finds similar $\Delta\Delta G$ values of 5–6 kJ/mol per salt bridge and a similar dependence on charges [10]. At zero ionic strength the stability decreases in the

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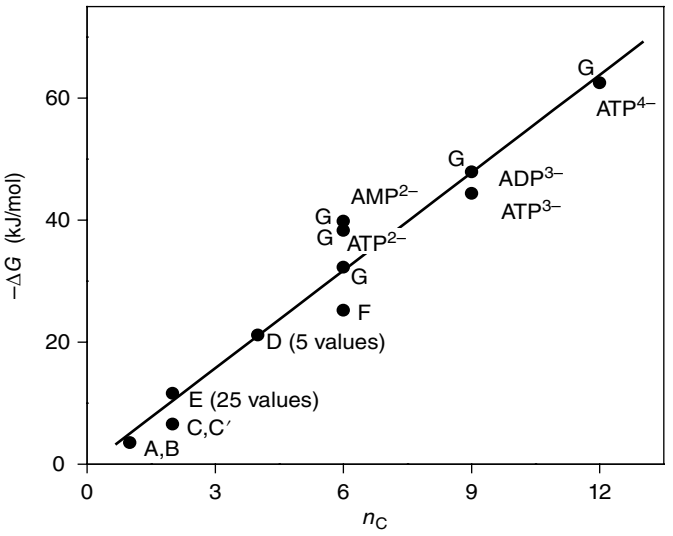
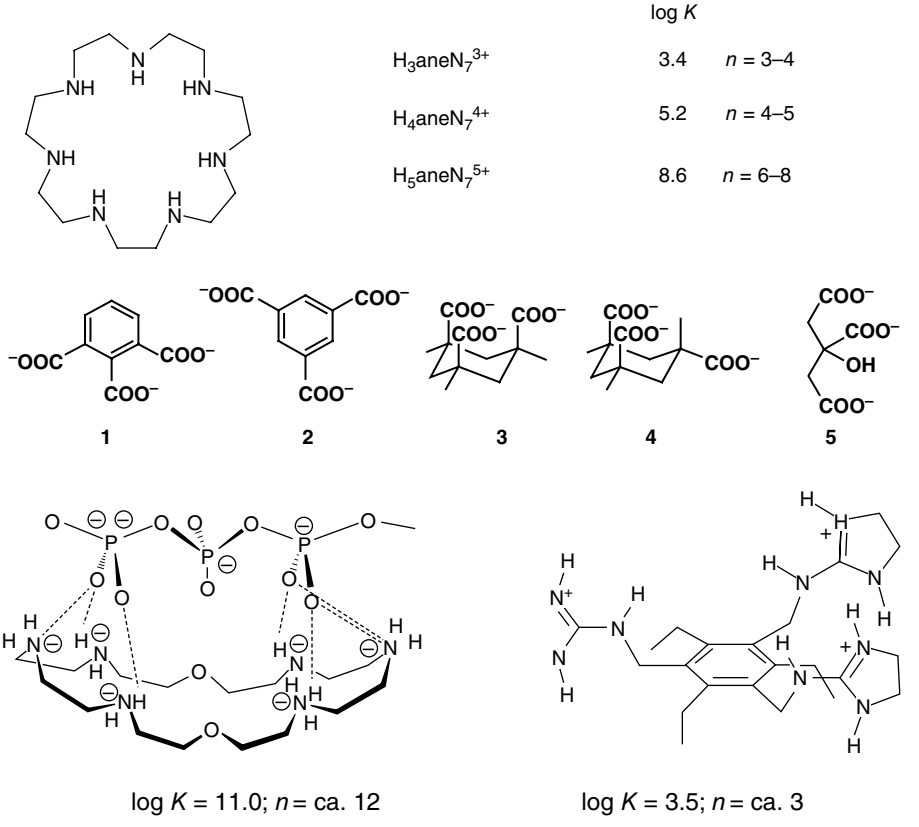


Figure 1.1 Additive ion pair contributions in a variety of complexes with a number n_C of salt bridges. From slope: average (5 ± 1) kJ/mol per salt bridge. A,B and C,C′ – complexes of a tetraphenolate cyclophane (4–) with Me_4N^+ and an azoniacyclophane (4+) with mono- and dianionic naphthalene derivatives; D – anionic (sulfonate or carboxylate) with cationic (ammonio) triphenylmethane derivatives; E – organic dianions with organic dications; F – cationic azamacrocycle (6+ charges) with aliphatic dicarboxylates; G – cationic azacrowns with adenosine mono-, di- and triphosphates. *Source*: Ref. [8]. Reproduced with permission of John Wiley and Sons.



Scheme 1.1 Complexation log *K* values of anions **1–5** with a macrocyclic amine as function of the degree of protonation of the amine; and ion pairing with some representative complexes; log *K* values in water; *n* is the estimated number of salt bridges.

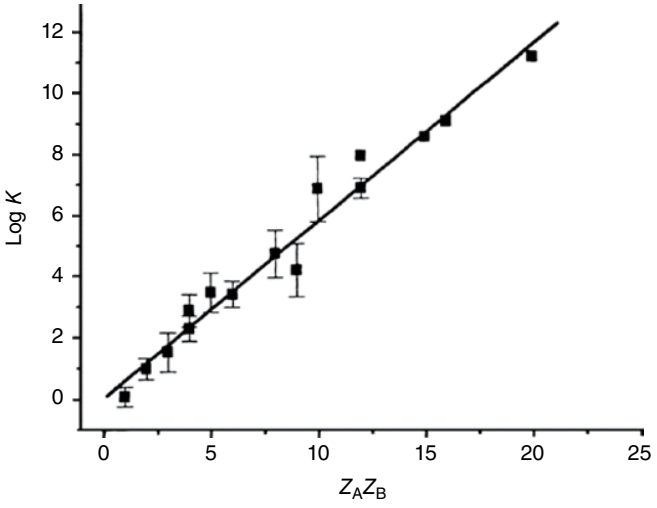


Figure 1.2 Ion pair association constants at zero ionic strength as a function of charge product, calculated for 203 ion pairs. *Source*: Ref. [8]. Reproduced with permission of John Wiley and Sons.

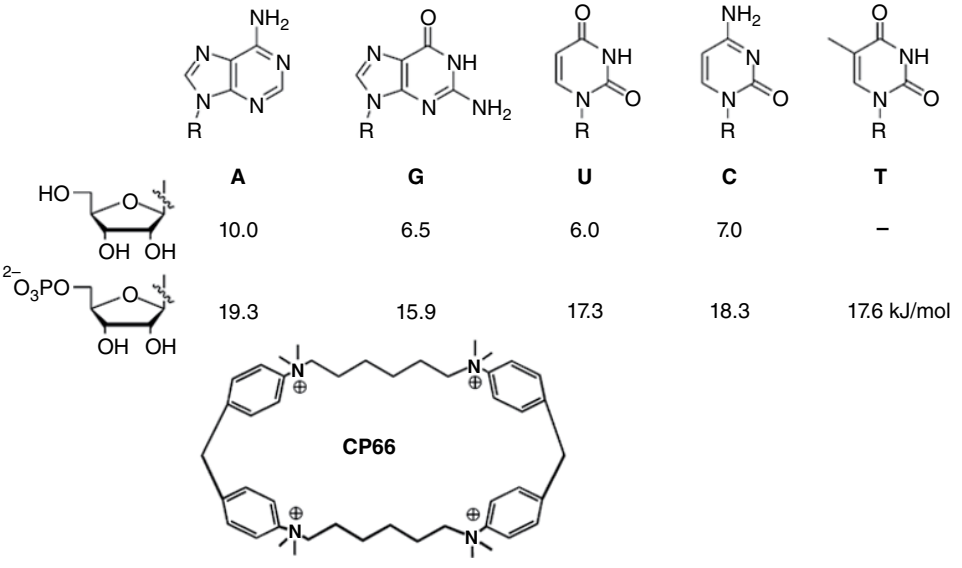
order $Ca^{2+} > Mg^{2+} > > Li^{+} > Na^{+} > K^{+}$ and can be described by Equation 1.2 [11]. Additivity is observed although ion pairing in water is determined entirely by entropic contributions[11], unless other contributions dominate [12].

$$\log K = 0.5z + \mathrm{A}/z \text{ (where A} = -0.24 \text{ for } Li^{+},\ -0.30 \text{ for } Na^{+},\ -0.43 \text{ for } K^{+}) \quad (1.2)$$

If there is more than one kind of interaction, Equation 1.3 applies. Often however, only one of the contributions is the same, like salt bridges in complexes of nucleotides with a positively charged host (Scheme 1.2) [13]. Additivity is then observed by the constant stability difference of $2 \times \Delta\Delta G \approx 10$ kJ/mol between complexes with charged nucleotides and neutral nucleosides. The 10 kJ/mol reflects the presence of two salt bridges between the phosphate dianion and the host ammonium center, which agrees with structural analyses by NMR spectroscopy.

$$\Delta G_t = n \cdot \Delta\Delta G_A + m \cdot \Delta\Delta G_B \quad (1.3)$$

The complexes shown in Scheme 1.2 exhibit constant single $\Delta\Delta G_A$ values only for the salt bridges, whereas the second contribution $\Delta\Delta G_B$ varies as a function of the different nucleobases. Figure 1.3 illustrates a case where both $\Delta\Delta G_A$ and $\Delta\Delta G_B$ remain constant, the latter reflecting cation–π interactions. In principle one could use Equation 1.3 to derive both $\Delta\Delta G_A$ and $\Delta\Delta G_B$, but more reliable values are obtained if for one interaction a $\Delta\Delta G$ value is used which is known from independent analyses, such as $\Delta\Delta G_A = 5$ kJ/mol for each salt bridge (see above). Then one observes a rather linear correlation with the number of phenyl units which shows a contribution of $\Delta\Delta G_B \approx 1.5$ kJ/mol for the single ^{+}N–π interaction [14].



Scheme 1.2 Complexation free energies ΔG of nucleotides and nucleosides with the cyclophane CP66.

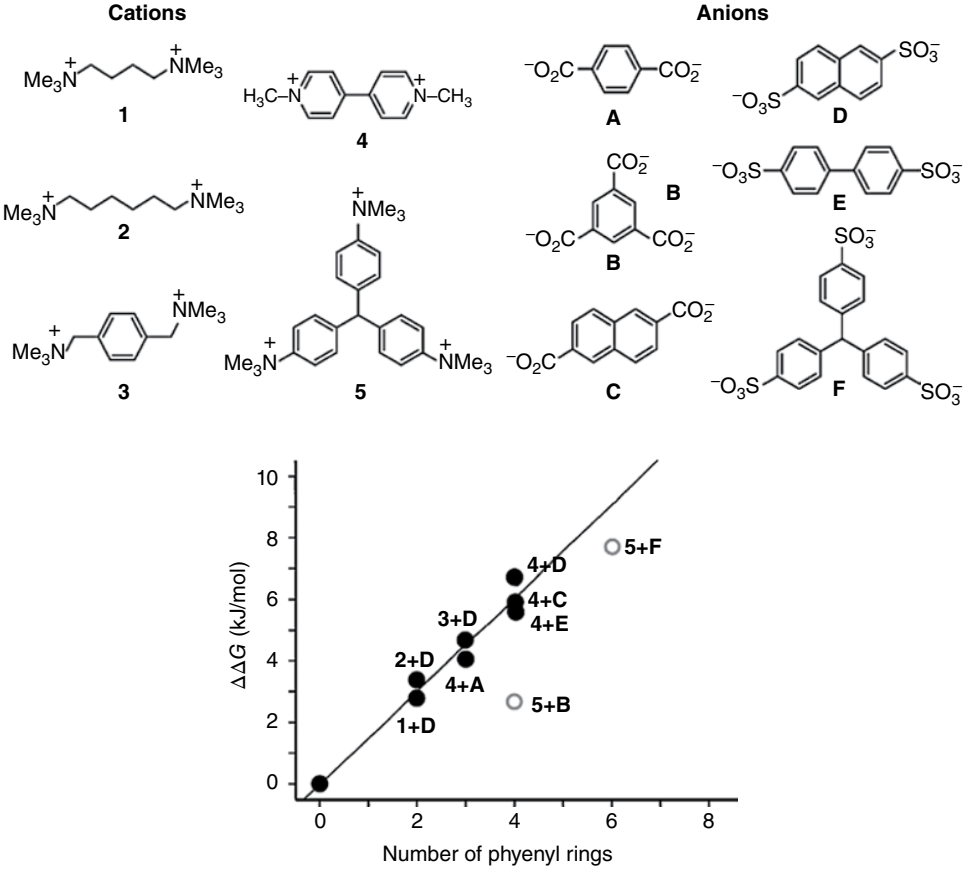


Figure 1.3 Ion pairs exhibiting both salt bridges and cation–π interactions; if $\Delta\Delta G_A = 5$ kJ/mol for each salt bridge are subtracted from ΔG_t of each complex. Outliers (open circles) are due to conformational mismatch. *Source*: Ref. [14]. Reproduced with permission of American Chemical Society.

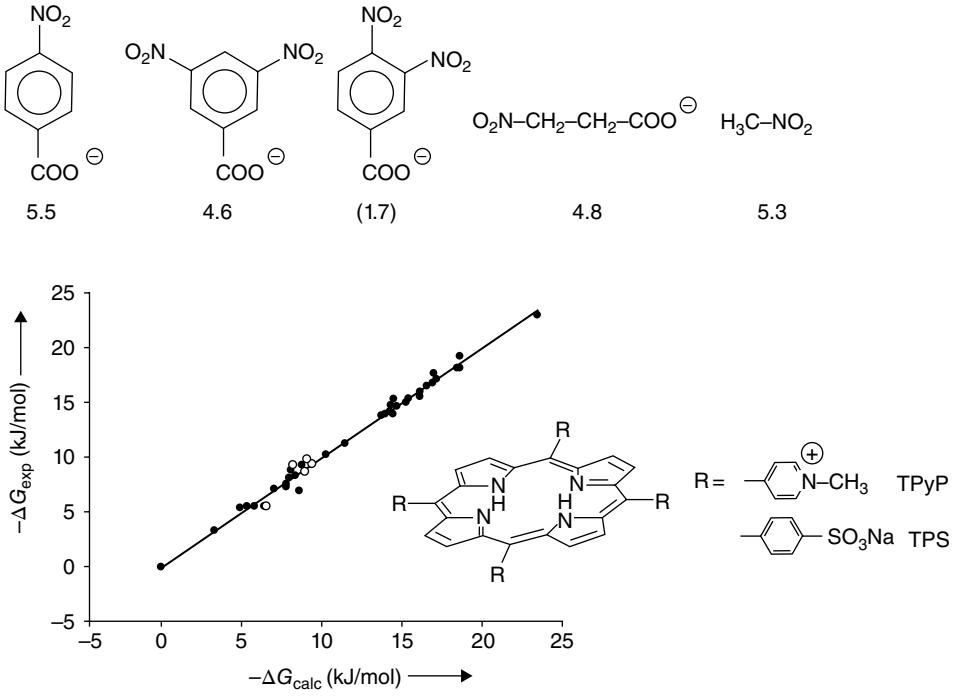


Figure 1.4 Additive $\Delta\Delta G_X$ increments in complexes of porphyrins bearing cationic or anionic substituents R in meso position (TPyP or TPS) in water, after deduction of 5 kJ/mol for ion pair contribution where applicable. $\Delta\Delta G_X$ increments in TPyP complexes for nitro substituents as an example (deviation for *ortho*-dinitro due to steric hindrance); correlation between measured complexation energies ΔG_{exp} and ΔG_{calc} calculated on the basis of experimentally determined averaged single contributions ΔG_S. Filled circles, complexes with TPyP; open circles, complexes with TPS. *Source*: Ref. [15]. Reproduced with permission of John Wiley and Sons.

The effect of nitro substituents on dispersive interactions is another example of additive energy contributions (Figure 1.4) [15,16]. Additivity with respect to substituent effects is observed in Hammett-type linear free energy relationship correlations; Figure 1.5 shows an example for hydrogen bonds with C—H bonds as donor and with hexamethylphosphoramide as acceptor [17].

1.3 Limitations of Additivity

1.3.1 Free Energy Values Δ*G* Instead of Enthalpic and Entropic Values Δ*H*, *T*Δ*S*

The examples shown above as well as most others in the literature rely on free energy values ΔG, although consideration of the corresponding ΔH and $T\Delta S$ parameters could shed more light on the underlying binding mechanisms. As pointed out earlier by Jencks, the empirical use of ΔG "avoids the difficult or insoluble problem of interpreting observed ΔH and $T\Delta S$ values for aqueous solution" [18]. Furthermore, according to Jencks, there

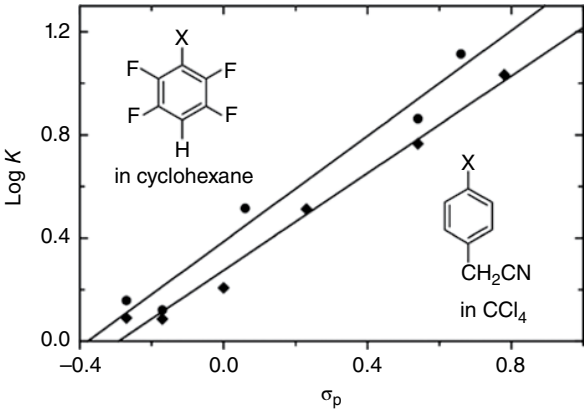


Figure 1.5 Hammett-type correlation of equilibria of hydrogen bonds with hexamethylphosphoramide as acceptor and para-substituted tetrafluorobenzenes or phenylacetonitriles as donor; log *K* versus Hammett substituent constants. *Source*: Ref. [17]. Reproduced with permission of John Wiley and Sons.

is often an additional "connection Gibbs energy, ΔG_S" (Equation 1.4) which he ascribed largely to changes in translational and rotational entropy. These connection ΔG_S can be either negative or positive and will be discussed as major liming factors for additivity below in the context of cooperativity and allostery.

$$\Delta G_t = \Delta G_A + \Delta G_B + \Delta G_S \tag{1.4}$$

The success of using free energy values instead of enthalpic and entropic values is in an essential part due to entropy–enthalpy compensation which has empirically been found to hold with many complexations, although it is theoretically not well-founded [19,20,21]. Another factor is that in typical supramolecular complexes the loss of translatory freedom is already paid by a single association step. The loss of rotational freedom upon complex formation has been experimentally [9] found to be smaller than theoretically expected (see below).

Entropy contributions pose particular problems, not only for the precise experimental determination, which in the past often relied on the temperature dependence of equilibrium constants (the *Van 'tHoff* method) instead of on more reliable calorimetry techniques. Also their theoretical interpretation is hampered by several factors, for instance because ΔS values depend on the choice of the standard concentration, in contrast to ΔH [8]. Configurational entropy, which refers also to solute motions has been addressed in several papers [22,23,24]. Data for the loss of translatory degrees of freedom in complex formation range from $T\Delta S = 3$ to 9 kJ/mol, and depend also on the reaction medium [25]. In multivalent associations this $T\Delta S$ penalty plays, as mentioned above, a minor role as it is paid already by a single interaction. For the loss of rotatory degrees of freedom in complex formation values from $T\Delta S = 1.5$ to 6 kJ/mol were proposed [26], which also should depend on the nature of the bond involved in the rotation [27]. Measurements of complexes involving an increasing number n of single bonds between two binding units furnished values of only $\Delta\Delta G = 0.5$ to 1.3 kJ/mol per single bond (e.g. from the slope in Figure 1.6) [9,28]. Similar small numbers have been found in

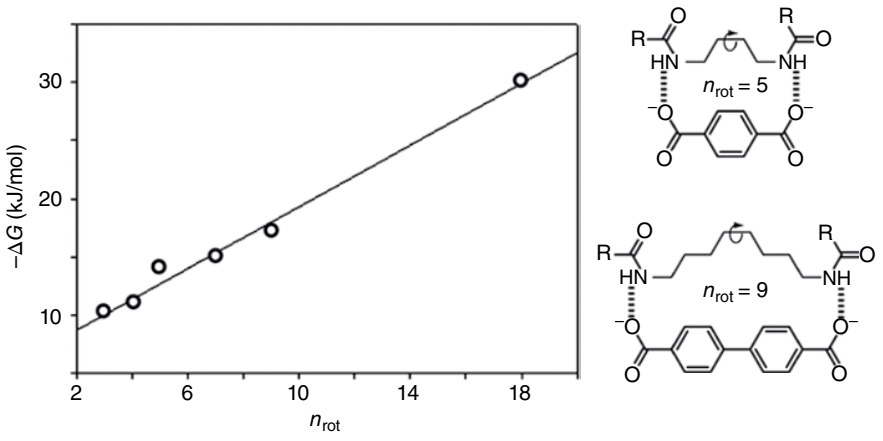
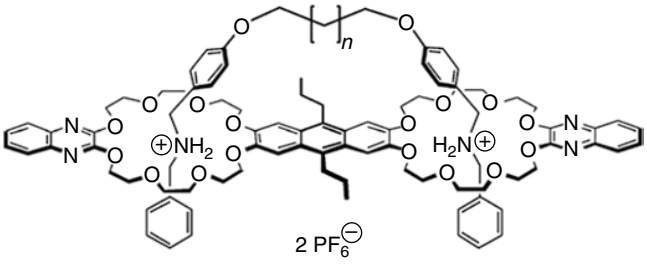


Figure 1.6 Free energies of complex formation between α,ω-diamides and α,ω-dicarboxylates in $CHCl_3$ as a function of the number of rotatable single bonds (n_{rot}) between the terminal amide and carboxylate functions. *Source*: Ref. [28]. Reproduced with permission of VCH/Wiley.

complexes involving peptide- ß-sheets [29], with calcium-EDTA complexes [30], and for example in the coordination of nickel or copper with either *trans*-1,2-diaminocyclohexane or the more flexible ethylene diamine [31]. In line with these rather small numbers it has been found that preorganization of a linker in host molecules has no or a small effect on supramolecular effective molarities [32,33].

1.3.2 Mismatch as Limitation of Additivity

The most obvious limitation for additivity of non-covalent interactions and therefore also for the lock-and-key principle is the necessary geometric fit between host and guest [34]. Insufficient fit between receptor and ligand is a major factor, in particular for a conformationally more rigid polyvalent entity [1]. The steric requirements for an optimal binding between host and guest depend on the nature of the non-covalent bonds. In particular, electrostatic interactions fall off with only with r^{-1} between binding sites whereas dispersive interactions fall off with r^{-6}. In addition, the latter interactions have no or only a small directional dependence, whereas for example the strength of hydrogen or halogen bonds depends on the orientation of donor and acceptor. Exceptions are molecular containers [35] in which the binding of substrates is in most cases controlled by the size of the portals. However, here as in other supramolecular complexes another important restriction is the presence of solvent molecules in a ligand-containing cavity, so that the guest molecule can only use a limited number of interactions which are possible, again depending on the binding mechanism. Thermal motions as well as vibrational and translatory freedom of movement of host and guest are also responsible for the limited fitting; moreover, the surfaces of interacting molecules are characterized by corners and dimples. Recent studies with cryptophanes composed of two bowl-shaped cyclotriveratrylene units showed large solvent molecules such as tetrachloroethane inside the cavity [36]. It has been found earlier [37] that for example some cryptophanes bind, say, chloroform better than methane, although methane fits geometrically as well in the cavity. An occupancy factor or packing coefficient (PC) of 0.886 was calculated for



Scheme 1.3 Complex with crown-ammonium pseudorotaxanes [39], with a very large affinity difference between spacer length of either $n = 0$ or $n = 1$.

the chloroform complex, similar to that in a closely packed crystal. For methane the occupancy factor amounts to a PC of only 0.35. These values are in the range with later systematic evaluations with many container- and capsule-type hosts [38], which were leading to generally observed 55 ± 9% occupancy of the space available.

Even small geometric changes can have a dramatic impact on the stability of supramolecular complexes, such as in recently described associations with crown-ammonium pseudorotaxanes [39] (Scheme 1.3). Here insertion of just one methylene group in the spacer leads to a drop from $K = 25\,000\ M^{-1}$ for the optimal spacer ($n = 0$) to $K = 1100\ M^{-1}$ with the longer spacer ($n = 1$), due to differences in both ΔH (−4.8 kJ/mol) and $T\Delta S$ (2.9 kJ/mol).

Frequently one interaction in a supramolecular complex is significantly larger than another one, which then can lead to an induced misfit. Figure 1.7 illustrates schematically the consequences for cyclodextrin complexes as an example [40]. Only in ideal situations like in Case I (Figure 1.7a) one can expect additivity (as for example with the nucleotide complexes in Scheme 1.2). In Case II (Figure 1.7b) the force between D and A is so strong that the second interaction is severely diminished, with an ensuing loss of additivity. Such situations have been seen for example with complexes of nucleotides and cyclodextrins, which bear a different number n of aminoalkyl substituents at the rim [41,42].

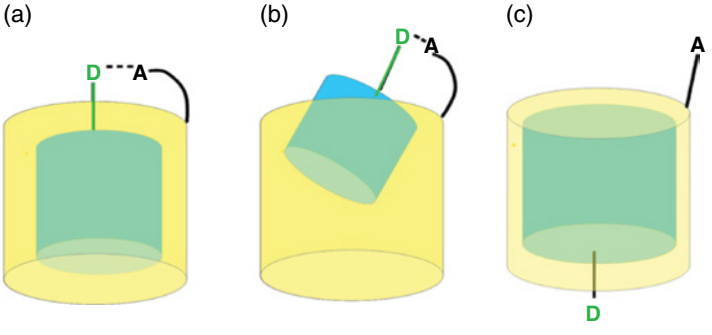
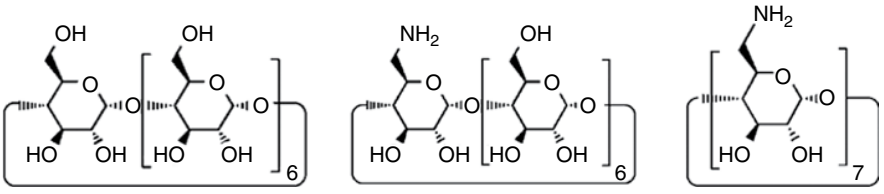
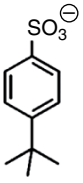


Figure 1.7 Schematic consequences of mismatch: (a) similar interaction in- and outside and sufficient matching (e.g. Case I); (b) stronger interaction outside (Case II); (c) stronger interaction inside cavity (e.g. Case III). *Source*: Ref. [40]. Reproduced with permission of Royal Society of Chemistry. See color section.



	CD0	CDI	CDII
5-AMP	11	24	29
5-CMP	—	17	24
ATP	—	28	32
	23	19.5	22.5

Scheme 1.4 Complexation free energies ΔG (kJ/mol) of ß-cyclodextrin derivatives bearing zero, one or seven charges at the rim (CD0, CDI, CDII) with AMP, ATP and *p-tert*-butylphenyl compounds. Data from Ref. [42].

With the monosubstituted cyclodextrin CDI ($n = 1$) the affinity increases from AMP to ATP by only $\Delta\Delta G = 4.7$ kJ/mol (Scheme 1.4), much less than expected by the possible increase of salt bridges between the phosphate residue and the CDI cation, and in contrast to observations with cyclophane complexes (Scheme 1.2). This indicates that the nucleoside residue seeks a sufficient contact with the CDI moiety, resulting in diminished ion pair contacts. Furthermore, there is a moderate selectivity with respect to the nucleobase, but the differences between AMP, GMP, CMP and UMP become smaller with the stronger binder CDII ($n = 7$), for example the $\Delta\Delta G$ between AMP and CMP diminishes from 7 to 4 kJ/mol (Scheme 1.4). This is the result of the then much stronger D$^{\cdots}$A salt bridge, which allows less contact between the cyclodextrin moiety and the nucleoside residue.

In Case III (Figure 1.7c) one interaction is so strong that the second one can barely materialize. The strong interaction of the butylphenyl residue in the cyclodextrins dominates the binding mode, and prohibits a contact between the anion and cation. This is obvious from the affinity with the positively charged host CDI which strikingly is even smaller in comparison with the neutral CD0, and from the negligible difference between CD0 and CDII complexes [42].

Stereoelectronic effects are also difficult to count as additive contribution, since they strongly depend on orientation, as shown for example for complexes between 1.10-diaza-crown and potassium ions [43]. Here, only after introduction of methyl groups at the nitrogen atoms are the lone pairs enforced towards a diequatorial orientation, and the binding energy increases to much larger affinity (Figure 1.8).

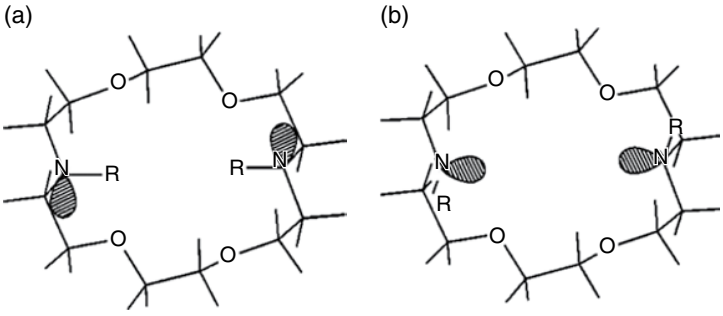


Figure 1.8 Stereoelectronics: the 1.10-diaza-crown with R = H (diaxial lone pair orientation, (a) binds K^+ ions with only $\Delta G = 10$ kJ/mol, with R = Me (diequatorial lone pair orientation, (b) ΔG increases to 26 kJ/mol (in methanol) *Source*: Ref. [43]. Reproduced with permission of John Wiley and Sons.

A similar situation holds for other directional enforcers, in particular for hydrogen bonds, and makes it difficult to simply summarize the number of interactions.

1.3.3 Medium Effects as Limiting Factor

Solvent effects can also significantly limit the possible additivity in multivalent complexes. First, they can decisively change the binding mechanism. Thus, dispersive interactions can be large in water, but are negligible in most organic solvents [16]. The energy for desolvation of host and guest prior to complex formation depends critically on the nature of binding elements, and thus can obscure additivity. In addition, solvophobic contributions can lead to a complete independence of specific non-covalent forces. In particular, water as medium, but also other solvents of low polarizability [44] can lead to dominating solvophobic forces. Especially cucurbituril hosts, which lack binding sites inside their cavity, complex with unsurpassed affinity with many ligands [45,46,47]. It has been shown that these cucurbiturils contain a sizeable number of water molecules which usually can exert only a few inter-water hydrogen bonds. If these are replaced by a suitable guest and freed to the bulk, they enjoy close to four hydrogen bonds. High energy water inside cavities is also present in for example cyclodextrins, cyclophanes, some tweezer or cleft hosts, and so on, and contributes to binding which is difficult to separate from direct non-covalent interactions [48] (Figure 1.9). Crystal structures of cyclodextrin hydrates have indicated the presence of such less coordinated water inside the cavity [49].

1.3.4 Strain and Induced Fit

Many, if not most complex formations occur with some conformational changes for maximizing the pertinent non-covalent interactions. Such an induced fit necessarily costs some strain energy, leading to weaker affinities than they would be if all possible interactions would be simply additive. This poses limits to the evaluation of additive single free energies from the observed total complexation free energies. Such strain effects play a particular role in cooperativity and allostery in multivalent complexes, which are dealt with in the following sections.

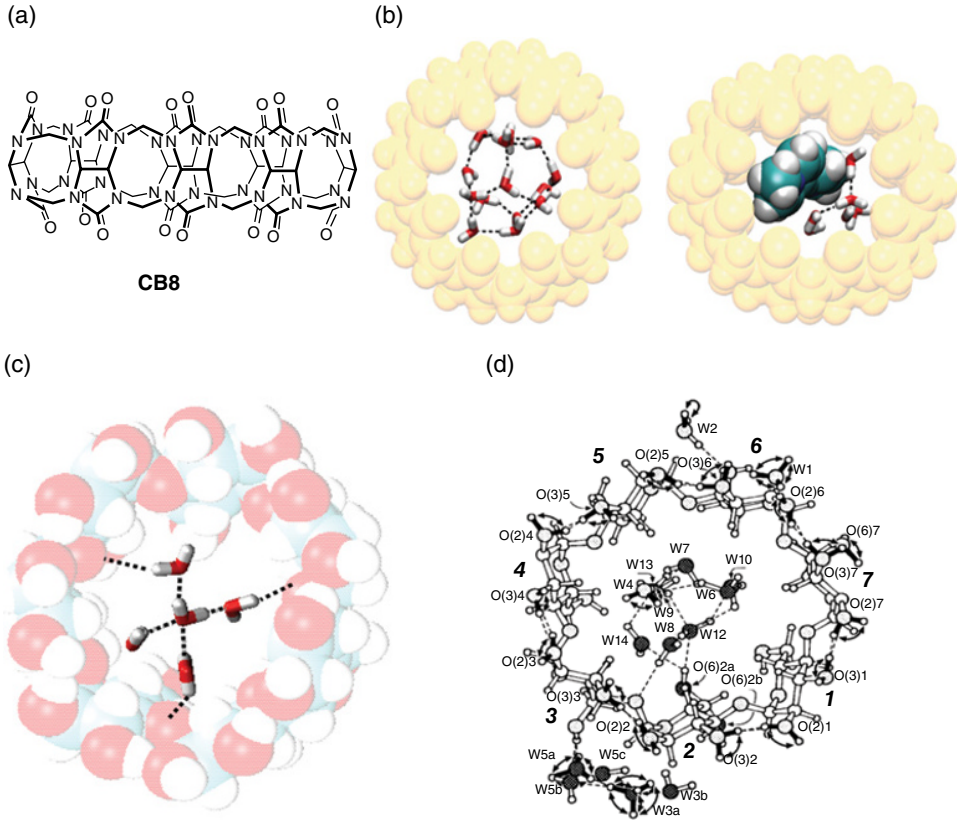
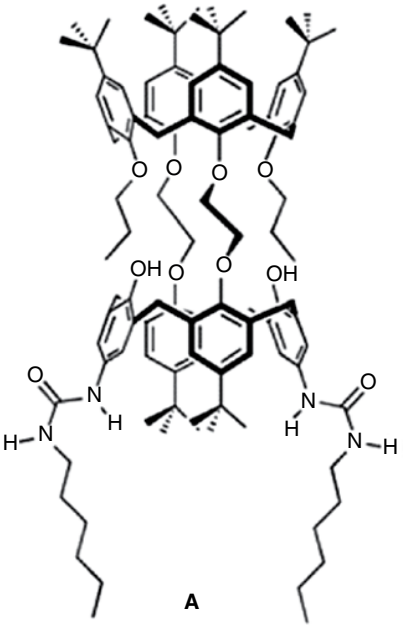


Figure 1.9 Examples of high energy water. (a) Cucurbit[8]uril (CB8) and 14 water molecules. (b) CB8 with viologen as guest and 6 water molecules in the cavity. (c) ß-Cyclodextrin with 5 water molecules, all from molecular dynamics simulations. *Source*: Ref. [48]. Reproduced with permission of John Wiley and Sons. (d) ß-Cyclodextrin dodecahydrate structure derived from neutron diffraction. *Source*: Ref. [49]. Reproduced with permission of American Chemical Society. See color section.

1.4 Cooperativity

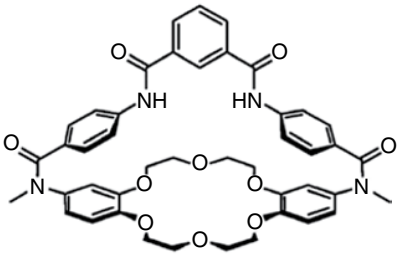
Positive cooperativity implies that the binding of one ligand to one of several binding sites in a receptor enhances the affinity at other sites, while negative cooperativity diminishes the affinity [1,4,5,50,51,52]. In classical allosteric systems this is due to conformational coupling between binding sites, as will be discussed in Section 1.5. Cooperativity also occurs if there are direct interactions between the complexed guest molecules. This is typical for ion pair complexation [53,54,55] where the electrostatic forces between anion and cation can lead to significantly enhanced binding constants K (Scheme 1.5). In Case A [56] the presence of Na^+ increases the value of K from 20 to 620 M^{-1}, in the crown ether host (Case B) the K increases from 50 to 470 M^{-1} in presence of Na^+ [57].

The cyclopeptide A shown in Scheme 1.6 binds $BuNMe_3X$ salts in chloroform for X = I with $K = 300\ M^{-1}$, while for the tosylate (X = OTs) a K increase by 10^4 was observed



A

K_{Na^+}/K_{free} = 31 for Cl^-
in $CDCl_3/CD_3CN$ (2:1)



B

K_{Na^+}/K_{free} = 9 for Cl^-
$CDCl_3/CD_3OD$ (9:1)

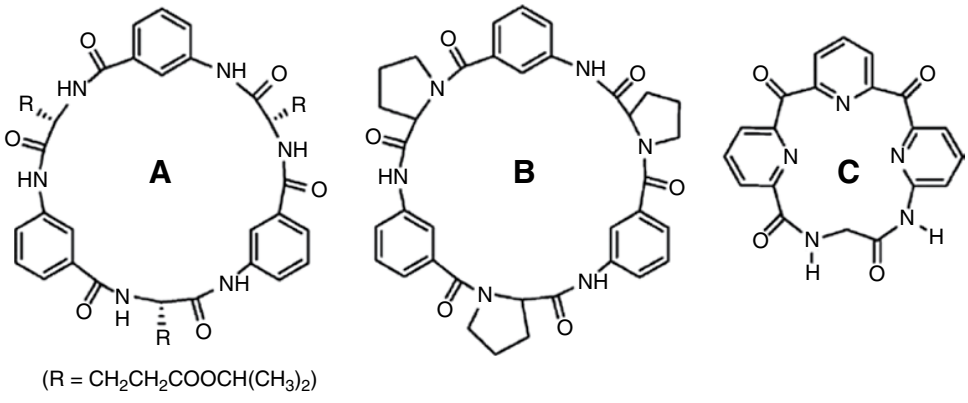
Scheme 1.5 Positive cooperativity: enhanced anion binding constants in heterotopic complexes.

compared with the iodide, explained also by a tosylate-induced conformational change of the host [58]. A related host B [59] binds very efficiently *N*-methyl-quinuclidinium iodide as ion pair in chloroform with $K = 8.3 \times 10^4\,M^{-1}$. With the host C a 260-fold affinity increase to $K = 1.8 \times 10^4\,M^{-1}$ was observed, with $^+H_3NCH(Bn)CO_2Me$ as the cation and nitrate as anion, while tetraalkylammonium salts bind weakly due the steric hindrance of the tetraalkyl residue, with for example $K = 70\,M^{-1}$ with nitrate as the anion [60].

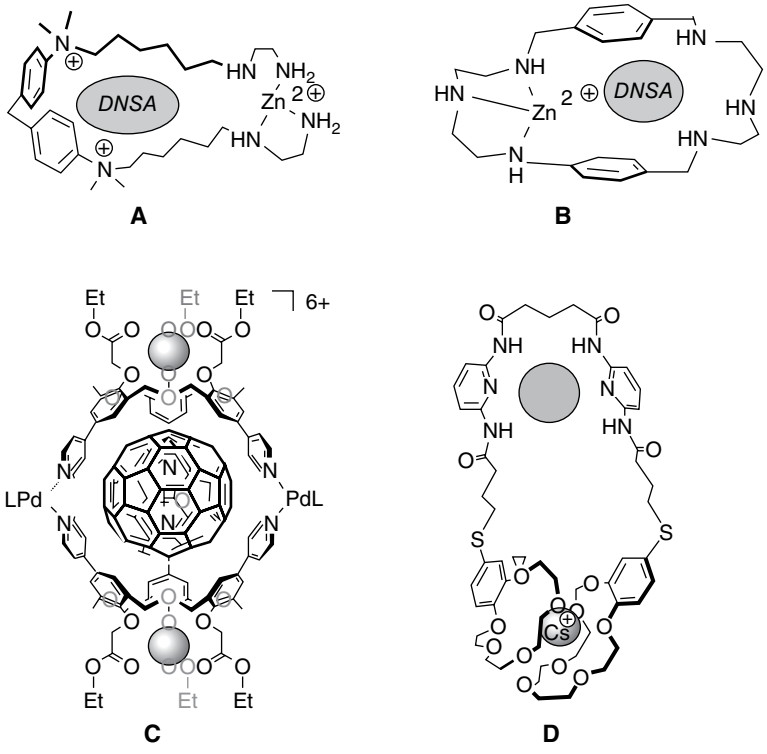
1.5 Allostery

Typical allosteric systems exhibit cooperativity due to conformational coupling between binding sites [61,62]. Case A shown in Scheme 1.6 [59] exemplifies that changes in flexible host structures may often play a large role in limitation of additivity rules. Extreme limitations of observable additivity occur in allosteric systems, which form a binding cavity only in the presence of strongly bound effector, such as metal ions in complexes A and B in Scheme 1.7. In complexes A and B the affinity of the fluorescent dye DNSA (dansylamide) in the absence of the zinc ion is so weak that it cannot be measured, so that the cooperativity ratio amounts to $K_{rel} = K_{Zn/0} > 100$ [63,64].

In complex C (Scheme 1.7) a conformational change induced by Li^+ ions leads to strong binding of [60]fullerene with $K = 2.1 \times 10^3\,M^{-1}$, in comparison with $K = 39\,M^{-1}$ without the metal [65]. A negative cooperativity is seen with Na^+, with $K_{rel} < 10$.

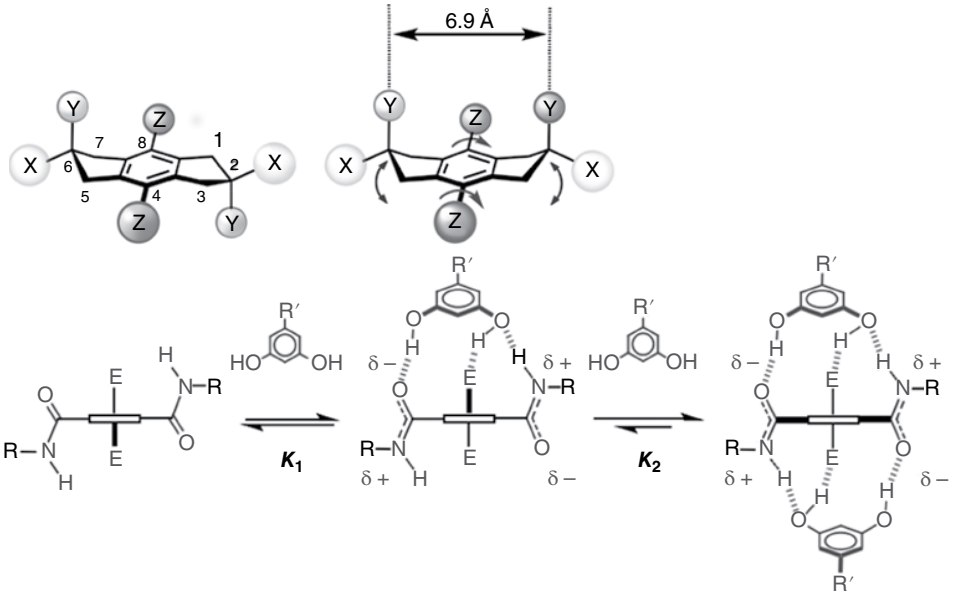


Scheme 1.6 Cyclopeptide hosts receptors with cooperativity between binding of anions and cations.



Scheme 1.7 Cooperativity with allosteric ditopic receptor complexes, see text.

The association of anions such as chloride with amide functions in complex D (Scheme 1.7) is significantly enhanced by complexation with Cs^+ ions, due to interaction with the crown ether units by a conformational rearrangement [66]. In *s*-hydrindacenes conformational changes of binding group orientation and polarity is observed upon association with substituted resorcinols, with a cooperativity ratio K_2/K_1 of up to 30 (Scheme 1.8) [67].



Scheme 1.8 Positive cooperativity in *s*-hydrindacene complexes: conformational changes lead to association with substituted resorcinols with a cooperativity ratio K_2/K_1 of up to 30 [68].

Conformational changes within a receptor, induced by an effector molecule, can lead to reinforced binding at different receptor locations [68]. In flexible proteins correlated rearrangement allows allosteric communication between different locations [69,70,71]; the ensuing entropic factors will limit the additivity of single binding contributions [72]. The host CER (Figure 1.10) exhibits a related allosteric complexation; it bears anionic binding groups attached at the end of cholic acid arms which by hydrophobic interactions between them fold back and complex by ion pairing 1,3,5-tris(amino methyl)

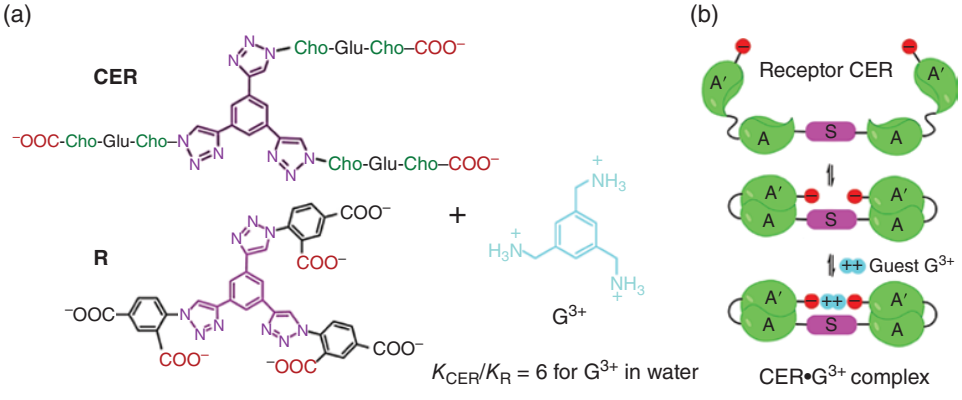


Figure 1.10 (a) Receptor CER with and R without steroidal arms, tricationic guest G^{3+}. (b) Hydrophobic interactions between the steroidal arms of CER preorganizes the CER for binding of guest G^{3+}. *Source*: Ref. [73]. Reproduced with permission of American Chemical Society. See color section.

benzene as guest G with $K = 138\,M^{-1}$, in comparison with only $24\,M^{-1}$ with a parent receptor R lacking the steroidal arms. Both enthalpic and entropic contributions are responsible for the different complexations [73].

Finally, we note that the decisive factor for the efficiency of allosteric systems with positive cooperativity is the conformational energy ΔG_C required for the formation of a suitable cavity for ligand binding in the absence of an effector. ΔG_C is usually dominated by an increase of strain in a folded conformer and/or by high energy solvents within a cavity. A recent analysis of the thermodynamics in synthetic allosteric systems exemplifies how the binding strength of an effector molecule at a second binding site must pay for the energy ΔG_C needed for the binding of the first substrate [74]. Additivity of the binding contributions can only be expected if ΔG_C could be determined independently. Negative cooperativity depends only on the difference $\Delta\Delta G_{A,B}$ between the binding energies at the two sites, which are enhanced or lowered by concomitant changes in ΔG_C. The often small efficiency of synthetic allosteric receptors [61,62], measured by the binding constant ratio $K_A/K_{A(B)}$, in which K_A refers to association of ligand A in the absence of the effector ligand B, and $K_{A(B)}$, is due to small ΔG_C values. Larger efficiency can be expected with increased ΔG_C values, for example by introduction of alkyl substituents in the *ortho*-position of pyridine in the often used [61,62] bipyridyl-based allosteric systems.

1.6 Conclusions

For efficient multivalent complexes it is desirable to preserve as much as possible additivity of all possible binding contributions. Ideally not only the affinity and therefore sensitivity but also the selectivity of such complexes is optimal if additivity of the single binding free energies is materialized. To what degree a geometric fit in the sense of the lock-and-key principle or preorganization is required depends first on the binding mechanism. The distance dependence of the interaction increases distinctly from electrostatic effects or ion pairing to dispersive forces. Mismatch between binding partners leads to a strong decrease of both affinity and selectivity particularly if the binding mechanism is characterized by a steeper distance dependence, and if the components are less flexible. Solvents can strongly influence the binding mechanisms; hydrophobic effects of high energy water inside cavities or clefts can make intermolecular binding contributions unimportant.

If one binding contribution is much stronger than others, the second interaction is often severely weakened due to mismatch; even a complete change of binding modes can occur. High selectivity combined with high affinity, which both require optimal fit, is difficult to attain if binding sites in a receptor are rigidly connected. In principle one can overcome this problem by flexible connections between a primary binding site securing high affinity with another site securing selectivity, provided such sites are available. If a multivalent complex should operate in for example a nanomolar solution the primary interactions should be worth about 50 kJ/mol, while at the secondary site values of, say, $\Delta G_X = 15$ and $\Delta G_Y = 5$ kJ/mol are enough to achieve a sizeable selectivity for distinction of two compounds X and Y ($\Delta\Delta G \approx 10$ kJ/mol or $K_X/K_Y \approx 100$).

In systems with positive cooperativity larger affinity than that predicted by additive single interactions is possible either by attractive forces between nearby bound

substrates, or in the classical case by conformational change at one site A induced by occupation at another site B. The efficiency of related allosteric systems can be defined as the ligand A concentration needed for complexation in the absence of the effector binding at B; it depends on the strain energy which would be needed to form an optimal conformation for binding A in the absence of occupation at B. Instead of conformational strain unfavorable solvents in cavities or clefts, such as high energy water, can enhance the efficiency of allosteric systems. It is hoped that the design of synthetic systems for, say, highly sensitive and selective new sensors as well as for drug design can be facilitated by taking into account some of the limitations and possibilities outlined in this chapter.

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2

Models and Methods in Multivalent Systems

Jurriaan Huskens

Molecular NanoFabrication Group, MESA+ Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, 7500 AE, Enschede, the Netherlands

2.1 Introduction

2.1.1 General Introduction

Self-assembly describes the non-covalent interaction between molecules, biomolecules, nanoparticles, and so on, that leads to larger structures with designed properties and functionalities [1,2,3,4,5]. The development of organic synthesis, which is the methodology development for the creation of new molecules based on covalent bonds, has led to a true revolution in chemistry that started more than a century ago. Literally millions of compounds have been made or can potentially be made based on the methods developed so far, and the field is still developing. One can hardly grasp the idea of taking all of these molecules and to assemble them into larger structures, particles, entities, based on non-covalent interactions: the possible combinations are unimaginable and are orders of magnitude larger in potential. Moreover, the products of self-assembly can in turn be organized in assemblies of a higher order, in a process called hierarchical self-assembly [6].

In chemistry, the notion of the infinite possibilities of self-assembly has grown in particular from the dawn of supramolecular chemistry in the 1960s and 1970s [2,7]. Nowadays, the concepts that have been and still are being developed in this area have pervaded all chemistry and materials science. In biology, self-assembly has been seen for decades as one of the major structuring and compartmentalization forces in nature. The current technological toolbox, with single-molecule techniques, *in-vivo* imaging, and so on, in particular developed within the chemical biology and nanotechnology arenas, now finally allows complete merging of the concepts and methods in the disciplines of chemistry and biology.

These trends put pressure on the current education of undergraduate and graduate students alike. The pervasion of supramolecular and many other concepts throughout materials science, nanotechnology, and chemical biology in its fullest breadth, causes students to need a vastly more multidisciplinary training, sometimes at the expense of monodisciplinary methodological approaches. For young scientists to play a role at the

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forefront of this immensely popular and interesting area of science, they need to be able to grasp broad concepts quickly, oversee the relationship of these concepts with and their importance for other disciplines, yet at the same time be able to go in depth on a subject when needed for a better fundamental understanding.

This chapter aims to provide an understanding of multivalent systems, based on the development of *models* for describing the binding behavior of these systems. In this context, a model is a complete set of species and the equilibria between them, in which the non-covalent bonds are formed in a stepwise manner, and all equilibria are coupled to equilibrium constants (and association and dissociation rate constants, when desired). The primary assumption made here is that a student or scientist will at some point get data on a non-covalently interacting system, and wants to understand what happens, without having to operate the proverbial "black box". Therefore, a direct and intimate connection is made here between real data and models, so that the development of more complex models can be undertaken from easier models and from a basic understanding of how these models can be implemented numerically. Therefore, this chapter starts (Section 2.2) with the development of the numerical treatment of equilibria and the most basic experimental method for assessing equilibria, the titration. This endeavor borrows pieces of knowledge from analytical chemistry and thermodynamics, as well as a bit of numerical mathematics (though hardly escaping the high school level). In practice, many of these methods have been used intensively in other areas of chemistry, such as coordination and analytical chemistry. This section is followed by a section (2.3) on basic models of multivalent systems, introducing the main concepts, such as effective molarity, and the differences compared with non-multivalent systems. Both solution and surface systems are discussed. Thereafter follows a section (2.4) with more specific, and sometimes more complicated, models.

2.1.2 Multivalent versus Cooperative Interactions

Multivalent systems are systems in which molecules interact with each other by more than one non-covalent interaction pair. The hallmark of a multivalent system is the occurrence of one (or more) *intra*molecular[i] interaction. The popularity of the field, and its importance for both chemical and biological systems, has spawned the publication of several reviews on the topic [8,9,10,11].

As will be explained in more detail below, multivalent systems often come with enhanced affinities, and these enhanced affinities have on occasions been taken as a sign of cooperativity. Therefore, a good definition of both terms, multivalency and cooperativity, is needed before the sense or nonsense of their applicability to a particular system can be evaluated.

We here take cooperativity (also called "allosterism" or "allosteric cooperativity") [12] to mean: the change of the affinity (lower or higher) of an interaction pair caused by the presence of a neighboring formed interaction pair. The textbook example of a (positively) cooperative system is hemoglobin, in which the uptake of oxygen by a heme binding site

[i] We explicitly take "intramolecular" to also encompass the non-covalent interaction of two ends of a molecular chain that has other non-covalent interactions present in the chain. All interactions are treated in a stepwise manner, and thus the focus on the formation of a particular interaction pair treats already formed non-covalent interactions in the system like normal, covalent bonds.