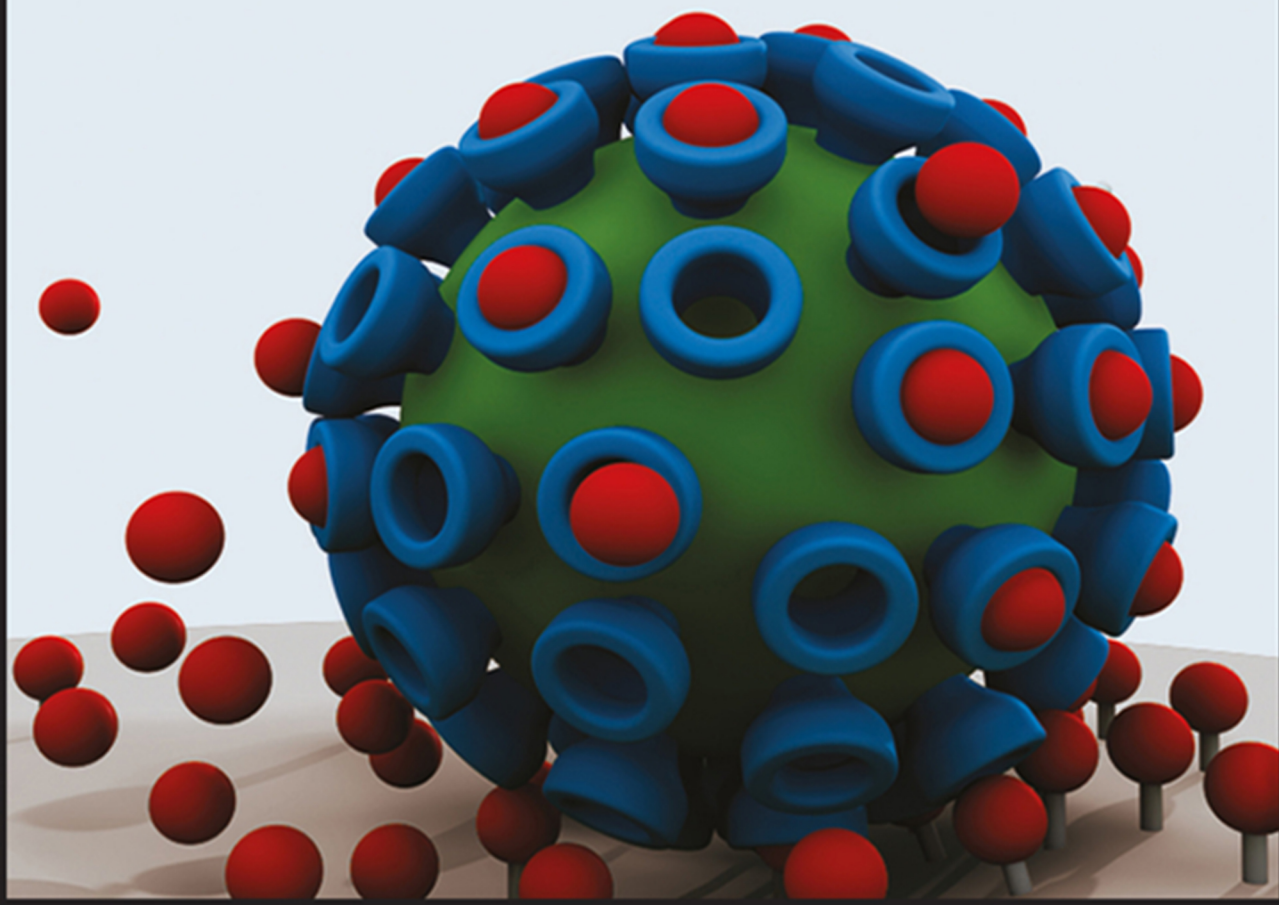


Edited by
Jurriaan Huskens, Leonard J. Prins,
Rainer Haag, Bart Jan Ravoo

Multivalency

Concepts, Research & Applications



Multivalency

Multivalency

Concepts, Research & Applications

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*

WILEY

This edition first published 2018
© 2018 John Wiley & Sons Ltd

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at <http://www.wiley.com/go/permissions>.

The right of Jurriaan Huskens, Leonard J. Prins, Rainer Haag, and Bart Jan Ravoo to be identified as the author(s) of the editorial material in this work has been asserted in accordance with law.

Registered Office(s)

John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office

9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty

In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. While the publisher and authors have used their best efforts in preparing this work, they make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives, written sales materials or promotional statements for this work. The fact that an organization, website, or product is referred to in this work as a citation and/or potential source of further information does not mean that the publisher and authors endorse the information or services the organization, website, or product may provide or recommendations it may make. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for your situation. You should consult with a specialist where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Cataloging-in-Publication Data

Names: Huskens, Jurriaan, 1968– editor.

Title: Multivalency : concepts, research & applications / edited by Professor Jurriaan Huskens,
University of Twente, Enschede, NL [and three others].

Description: First edition. | Hoboken, NJ : Wiley, 2018. | Includes bibliographical references and index. |

Identifiers: LCCN 2017029790 (print) | LCCN 2017039365 (ebook) | ISBN 9781119143475 (pdf) |

ISBN 9781119143499 (epub) | ISBN 9781119143468 (cloth)

Subjects: LCSH: Valence (Theoretical chemistry) | Multivalent molecules.

Classification: LCC QD469 (ebook) | LCC QD469 .M75 2018 (print) | DDC 541/.224–dc23

LC record available at <https://lcn.loc.gov/2017029790>

Cover design by Wiley

Cover image: Image provided by Rainer Haag

Set in 10/12pt Warnock by SPi Global, Pondicherry, India

Contents

List of Contributors *xi*

Foreword *xv*

Preface *xvii*

Part I General Introduction to Multivalent Interactions *1*

1 Additivity of Energy Contributions in Multivalent Complexes *3*

Hans-Jörg Schneider

1.1 Introduction *3*

1.2 Additivity of Single Interactions – Examples *3*

1.3 Limitations of Additivity *7*

1.3.1 Free Energy Values ΔG Instead of Enthalpic and Entropic Values ΔH , $T\Delta S$ *7*

1.3.2 Mismatch as Limitation of Additivity *9*

1.3.3 Medium Effects as Limiting Factor *12*

1.3.4 Strain and Induced Fit *12*

1.4 Cooperativity *13*

1.5 Allostery *14*

1.6 Conclusions *17*

References *18*

2 Models and Methods in Multivalent Systems *23*

Jurriaan Huskens

2.1 Introduction *23*

2.1.1 General Introduction *23*

2.1.2 Multivalent versus Cooperative Interactions *24*

2.2 Numerical Data Analysis *25*

2.2.1 Model Simulations Using a Spreadsheet Approach *26*

2.2.2 Setting Up and Assessing Titrations *30*

2.2.3 Using Spreadsheet Simulations to Fit Experimental Data to a Model *36*

2.3 Models for Multivalent Systems *41*

2.3.1 The Simplest Multivalent System: A 1:1 Complex with Two Interaction Sites *41*

2.3.2 Multivalent Binding at Surfaces *46*

2.4	Special Multivalent Systems	53
2.4.1	Increasing the Valency of Interfacial Assemblies: Dendrimers, Oligomers, and Polymers	53
2.4.2	Heterotropic Interactions	58
2.4.3	Kinetics and Dynamics	63
2.5	Conclusions	68
	Acknowledgments	68
	References	68
3	Design Principles for Super Selectivity using Multivalent Interactions	75
	<i>Tine Curk, Jure Dobnikar, and Daan Frenkel</i>	
3.1	Introduction	75
3.1.1	Background: Ultra-sensitive Response	75
3.2	Super Selectivity: An Emergent Property of Multivalency	78
3.3	Multivalent Polymer Adsorption	84
3.4	Which Systems are Super Selective?	86
3.4.1	Rigid Geometry Interactions	86
3.4.2	Disordered Multivalency	87
3.5	Design Principles for Super-Selective Targeting	90
3.6	Summary: It is interesting, but is it useful?	93
	Appendix 3.A: What Is Effective Molarity?	95
	Acknowledgements	98
	References	98
4	Multivalency in Biosystems	103
	<i>Jens Darnedde</i>	
4.1	Introduction	103
4.2	Cell–Cell Adhesion	104
4.2.1	Homotypic Interactions, Cadherins Keep Cells Together	105
4.2.2	Selectins, Heterotypic Cell Adhesion to Fight Infections	106
4.2.3	Bacterial Adhesion by FimH	108
4.3	Phase Transition, Multivalent Intracellular Assemblies	109
4.4	Multivalency in the Fluid Phase, Pathogen Opsonization	111
4.5	Conclusion	113
	Acknowledgment	113
	References	114
Part II Multivalent Systems in Chemistry		121
5	Multivalency in Cyclodextrin/Polymer Systems	123
	<i>Akihito Hashidzume and Akira Harada</i>	
5.1	Introduction	123
5.2	General Perspectives of Multivalency in Cyclodextrin/Polymer Systems	125
5.3	Typical Examples of Multivalency in Cyclodextrin/Polymer Systems	126

5.3.1	Formation of Polymer Aggregates from Cyclodextrin-Polymers and Guest-Polymers	126
5.3.2	Selectivity of Interaction Enhanced by Multivalency	127
5.3.3	Self-Healable Hydrogels Based on Multivalency	134
5.4	Summary and Outlook	136
	Acknowledgments	136
	References	138
6	Cucurbit[n]uril-Mediated Multiple Interactions	143
	<i>Zehuan Huang and Xi Zhang</i>	
6.1	Introduction to Cucurbit[n]uril Chemistry	143
6.2	Heteroternary Complexes	143
6.3	Homoternary Complexes	146
6.4	Conclusions	150
	References	150
7	Multivalency as a Design Criterion in Catalyst Development	153
	<i>Paolo Scrimin, Maria A. Cardona, Carlos M. León Prieto, and Leonard J. Prins</i>	
7.1	Introduction	153
7.2	Formation of Enzyme-Like Catalytic Pockets	154
7.3	Cooperativity Between Functional Groups	157
7.4	Mechanistic Effects	161
7.5	The Dendritic Effect in Multivalent Nanozymes	164
7.5.1	Peptide-Based Dendrimers for the Cleavage of Phosphodiesterases	166
7.5.2	Catalytic 3D SAMs on Au NPs	168
7.6	Multivalent Catalysts and Multivalent Substrates	170
7.7	Conclusions	172
	Acknowledgements	174
	References	174
8	Multivalent Molecular Recognition on the Surface of Bilayer Vesicles	177
	<i>Jens Voskuhl, Ulrike Kauscher, and Bart Jan Ravoo</i>	
8.1	Introduction	177
8.2	Molecular Recognition of Vesicles	179
8.2.1	Metal Coordination	180
8.2.2	Light Responsive Interactions	184
8.2.3	Hydrogen Bonding and Electrostatic Interactions	185
8.3	Biomimetic Vesicles	188
8.3.1	Vesicles as Multivalent Platforms	188
8.3.2	Membrane Fusion	193
8.4	Vesicle-based Supramolecular Materials	196
8.4.1	Hydrogels	196
8.4.2	Immobilization of Vesicles	198
8.4.3	Nanoparticles and Nanocontainers	198
8.5	Conclusion	201
	Acknowledgment	201
	References	201

Part III Multivalent Systems in Biology 205**9 Blocking Pathogens by Multivalent Inhibitors 207***Sumati Bhatia, Benjamin Ziem, and Rainer Haag*

- 9.1 Introduction 207
- 9.2 Design of Multivalent Ligand Architectures 209
- 9.3 Multivalent Carbohydrate Ligands 212
- 9.4 Scaffold Architecture 215
 - 9.4.1 Linear and Dendritic Scaffolds 215
 - 9.4.2 Multivalent Gold Nanoparticles 218
 - 9.4.3 2D Platforms 220
- 9.5 Nano- and Microgels for Pathogen Inhibition 222
- 9.6 Conclusion 223
- Acknowledgments 224
- References 224

10 Multivalent Protein Recognition Using Synthetic Receptors 229*Akash Gupta, Moumita Ray, and Vincent M. Rotello*

- 10.1 Introduction 229
- 10.2 Structural Properties of Protein Surfaces 229
 - 10.2.1 Protein–Protein Interfacial Areas 229
 - 10.2.2 Chemical Nature of the Protein–Protein Interface 230
 - 10.2.3 “Hot Spots” 230
 - 10.2.4 O-Ring Structure 232
- 10.3 Synthetic Receptors for Protein Surface Recognition 232
 - 10.3.1 Porphyrin Scaffolds for Protein Surface Recognition 232
 - 10.3.2 Protein Surface Recognition Using Molecular Tweezers 238
 - 10.3.3 Calixarene Scaffolds for Protein Surface Recognition 240
 - 10.3.4 Recognition of Protein Surfaces Using Nanoparticles 243
 - 10.3.4.1 Nanoparticles as Protein Mimics 244
 - 10.3.4.2 Regulating the Structure and Function of Proteins Using Nanoparticles 246
 - 10.3.4.3 Nanoparticle-based Protein Sensors 250
- 10.4 Future Perspective and Challenges 254
- Acknowledgment 257
- References 257

11 Multivalent Calixarenes for the Targeting of Biomacromolecules 263*Francesco Sansone and Alessandro Casnati*

- 11.1 Introduction 263
- 11.2 Binding to Proteins and Enzymes 266
- 11.3 Recognition of Carbohydrate Binding Proteins (Lectins) 273
- 11.4 Binding Polyphosphates, Oligonucleotides and Nucleic Acids 279
- 11.5 Conclusions 284
- Acknowledgements 285
- References 285

12	Cucurbit[n]uril Assemblies for Biomolecular Applications	291
	<i>Emanuela Cavatorta, Luc Brunsveld, Jurriaan Huskens, and Pascal Jonkheijm</i>	
12.1	Introduction	291
12.2	Molecular Recognition Properties of CB[n]	293
12.2.1	Interactions with the Carbonyl Portals of CB[n]	293
12.2.2	Release of High Energy Water Molecules from the CB[n] Cavity	295
12.2.3	Enthalpy-driven Hydrophobic Effect for CB[n]	295
12.2.4	Enthalpy-driven Hydrophobic Effect for CB[8] Heteroternary Complexes	297
12.3	Control Over the Binding Affinity with CB[n]	299
12.4	CB[n] Recognition of Amino Acids, Peptides, and Proteins	301
12.5	CB[n] for Bioanalytical and Biomedical Applications	305
12.5.1	CB[n]-mediated Assembly of Bioactive Polymers and Hydrogels	305
12.5.2	CB[n]-mediated Assembly of Bioactive Nanoparticles	307
12.5.3	CB[n]-mediated Assembly on Bioactive Surfaces	313
12.6	Conclusions and Outlook	317
	Acknowledgment	319
	References	319
13	Multivalent Lectin–Glycan Interactions in the Immune System	325
	<i>João T. Monteiro and Bernd Lepenies</i>	
13.1	Introduction	325
13.2	Targeting Innate Immunity to Shape Adaptive Immunity	327
13.3	C-type Lectin Receptors	328
13.3.1	Multivalent Glycoconjugates Targeting DC-SIGN	331
13.3.2	Multivalent Glycoconjugates Targeting Other CLRs	331
13.4	Galectins	332
13.5	Siglecs	334
13.6	Conclusions	335
	Acknowledgment	335
	References	335
14	Blocking Disease Linked Lectins with Multivalent Carbohydrates	345
	<i>Marjon Stel and Roland J. Pieters</i>	
14.1	Introduction	345
14.2	Haemagglutinin	347
14.3	LecA	349
14.4	LecB	354
14.5	Galectins	358
14.6	Concanavalin A	362
14.7	Cholera Toxin	366
14.8	Propeller Lectins	367
14.9	Conclusion	371
	Acknowledgements	371
	References	371
	Index	381

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 $K \approx 10 \text{ M}^{-1}$ per salt bridge for typical complexes where the affinity depends as expected on the degree of protonation [7].

$$\Delta G_t = n \cdot \Delta\Delta G_i \quad (1.1)$$

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

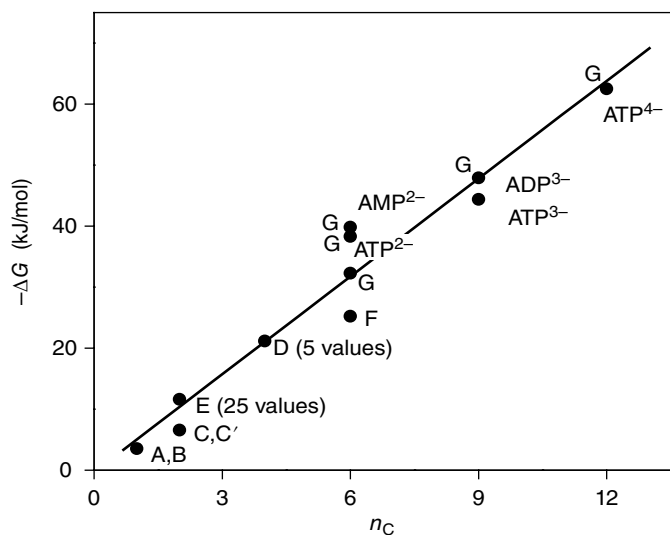
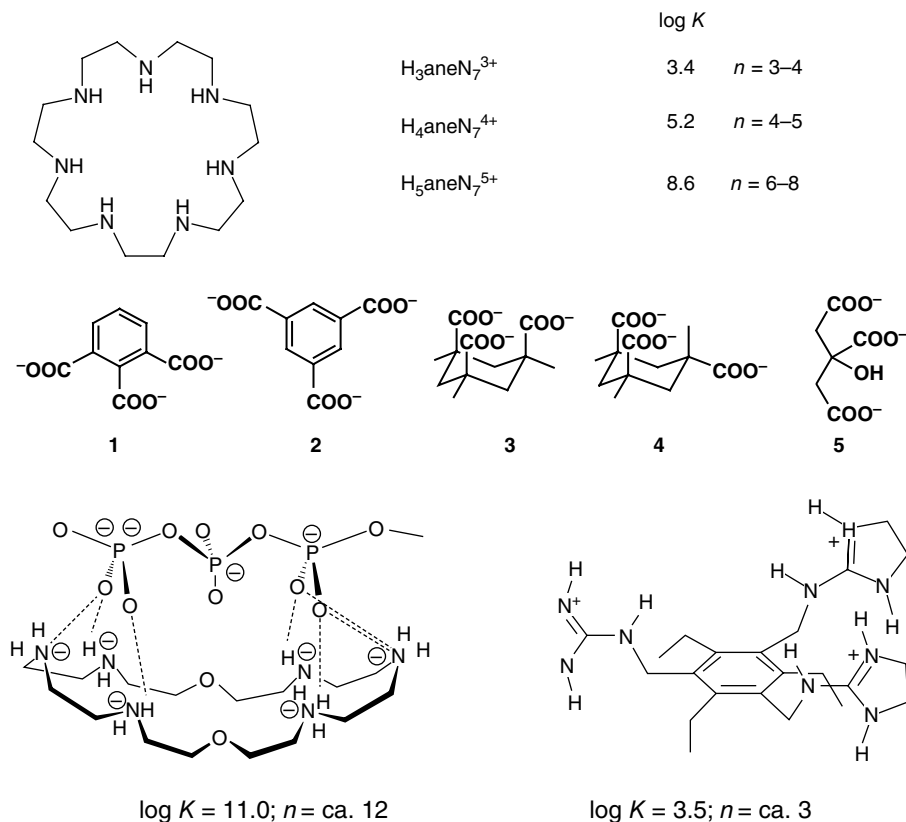


Figure 1.1 Additive ion pair contributions in a variety of complexes with a number n_C of salt bridges. From slope: average (5 ± 1) kJ/mol per salt bridge. A,B and C,C' – complexes of a tetraphenolate cyclophane (4–) with Me_4N^+ and an azoniacyclopentane (4+) with mono- and dianionic naphthalene derivatives; D – anionic (sulfonate or carboxylate) with cationic (ammonio) triphenylmethane derivatives; E – organic dianions with organic dications; F – cationic azamacrocyclic (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.

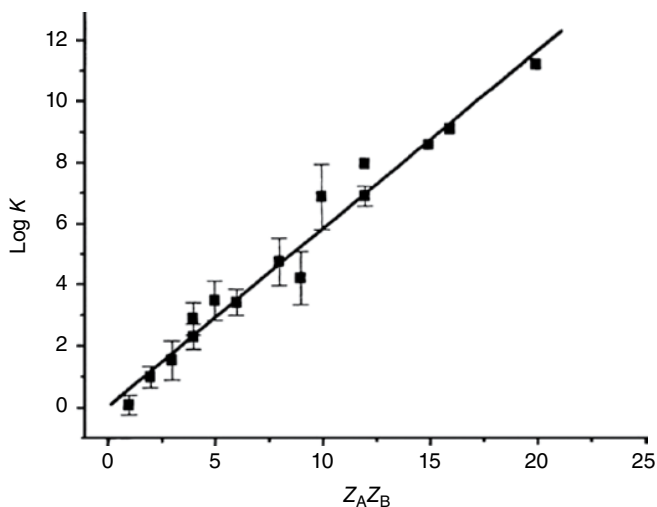


Figure 1.2 Ion pair association constants at zero ionic strength as a function of charge product, calculated for 203 ion pairs. *Source:* Ref. [8]. Reproduced with permission of John Wiley and Sons.

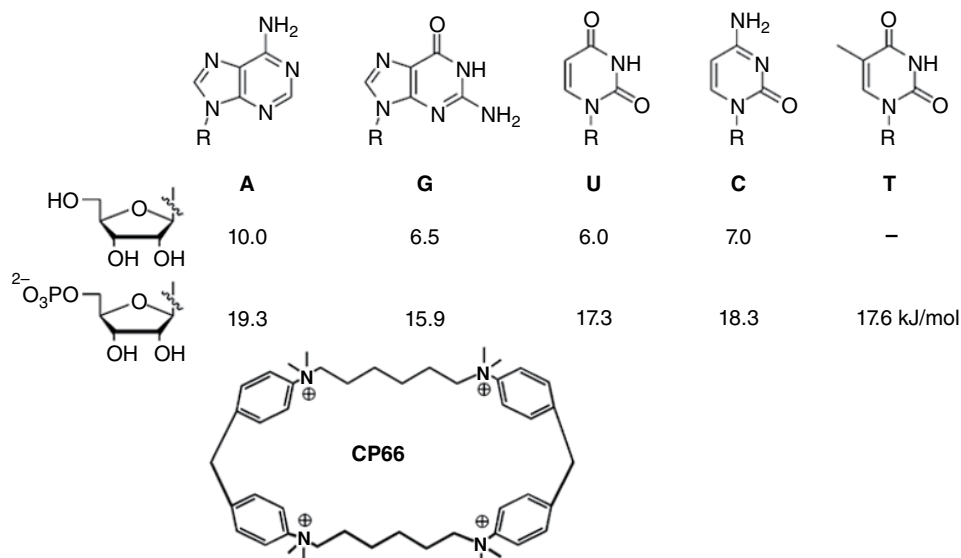
order $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Li}^+ > \text{Na}^+ > \text{K}^+$ and can be described by Equation 1.2 [11]. Additivity is observed although ion pairing in water is determined entirely by entropic contributions [11], unless other contributions dominate [12].

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

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 $2 \times \Delta\Delta G \approx 10 \text{ kJ/mol}$ 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 $\Delta\Delta G_A = 5 \text{ kJ/mol}$ for each salt bridge (see above). Then one observes a rather linear correlation with the number of phenyl units which shows a contribution of $\Delta\Delta G_B \approx 1.5 \text{ kJ/mol}$ for the single $^+\text{N}-\pi$ interaction [14].



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

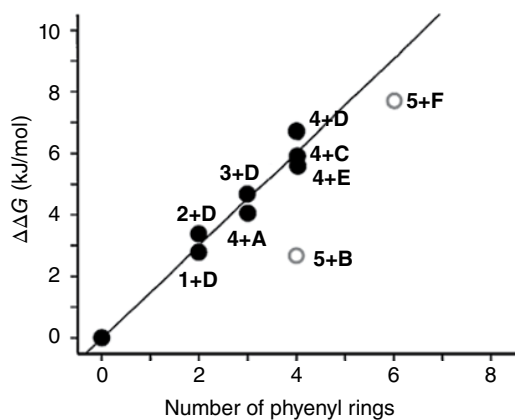
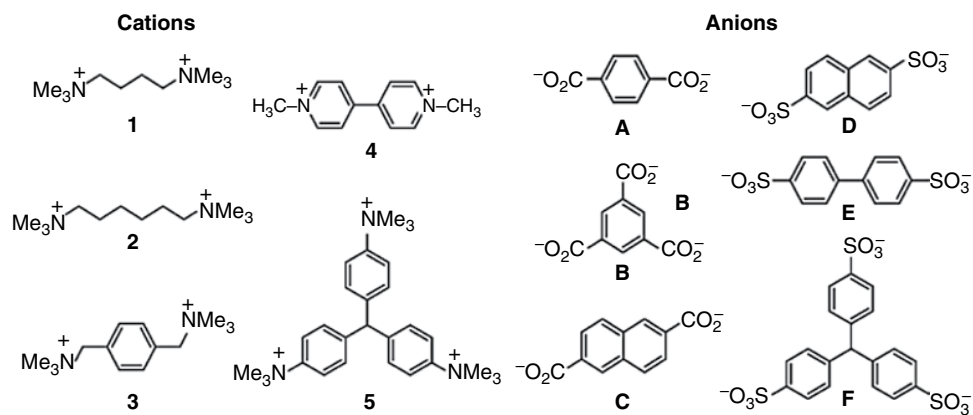


Figure 1.3 Ion pairs exhibiting both salt bridges and cation- π interactions; if $\Delta\Delta G_A = 5$ kJ/mol for each salt bridge are subtracted from ΔG_t of each complex. Outliers (open circles) are due to conformational mismatch. Source: Ref. [14]. Reproduced with permission of American Chemical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

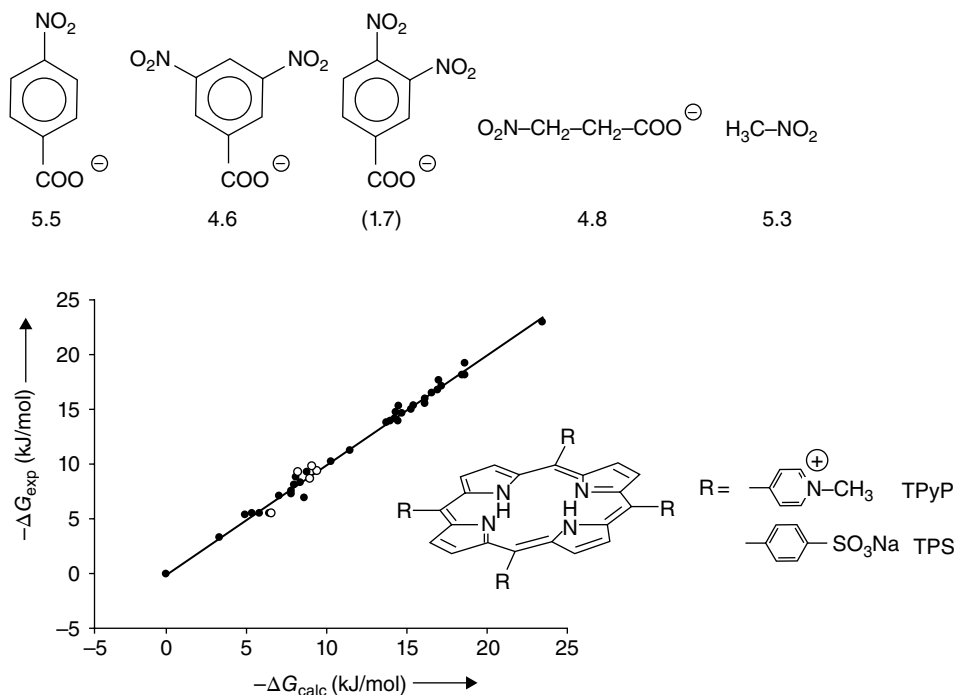


Figure 1.4 Additive $\Delta\Delta G_x$ increments in complexes of porphyrins bearing cationic or anionic substituents R in meso position (TPyP or TPS) in water, after deduction of 5 kJ/mol for ion pair contribution where applicable. $\Delta\Delta G_x$ increments in TPyP complexes for nitro substituents 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 ΔG Instead of Enthalpic and Entropic Values ΔH , $T\Delta S$

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

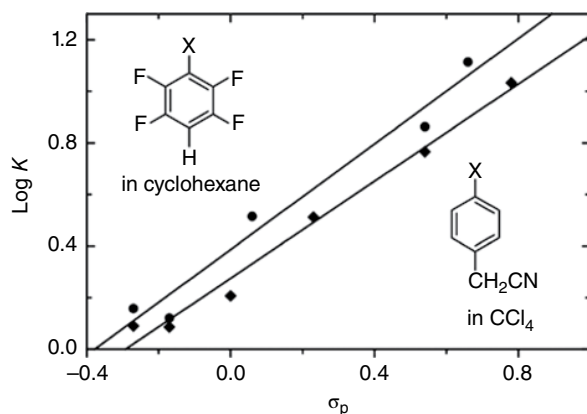


Figure 1.5 Hammett-type correlation of equilibria of hydrogen bonds with hexamethylphosphoramide as acceptor and para-substituted tetrafluorobenzenes or phenylacetone nitriles as donor; $\log K$ versus Hammett substituent constants. Source: Ref. [17]. Reproduced with permission of John Wiley and Sons.

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 limiting factors for additivity below in the context of cooperativity and allostery.

$$\Delta G_t = \Delta G_A + \Delta G_B + \Delta G_S \quad (1.4)$$

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

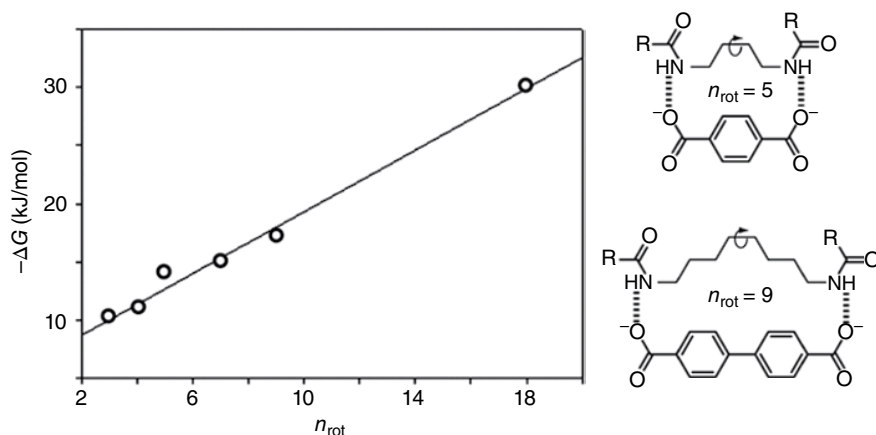
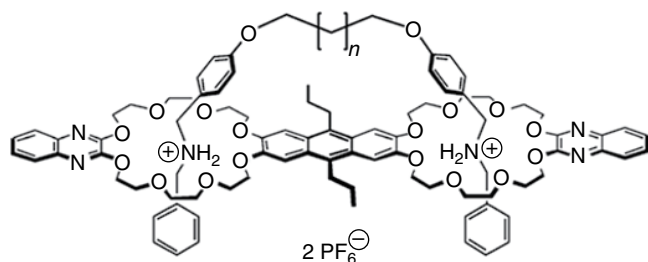


Figure 1.6 Free energies of complex formation between α,ω -diamides and α,ω -dicarboxylates in CHCl_3 as a function of the number of rotatable single bonds (n_{rot}) between the terminal amide and carboxylate functions. Source: Ref. [28]. Reproduced with permission of VCH/Wiley.

complexes involving peptide- β -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 cyclotrimeratrylene 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 $55 \pm 9\%$ 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 $K = 25\,000\text{ M}^{-1}$ for the optimal spacer ($n=0$) to $K = 1100\text{ M}^{-1}$ 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].

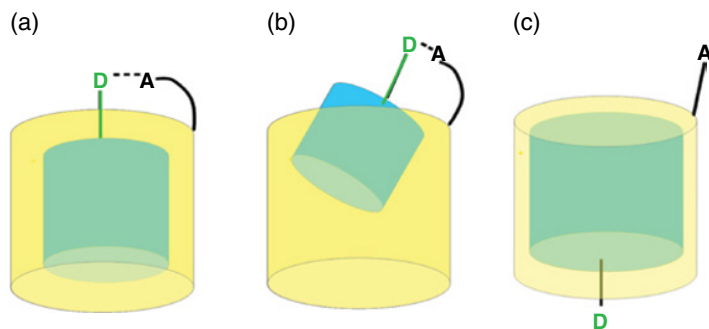


Figure 1.7 Schematic consequences of mismatch: (a) similar interaction in- and outside and sufficient matching (e.g. Case I); (b) stronger interaction outside (Case II); (c) stronger interaction inside cavity (e.g. Case III). Source: Ref. [40]. Reproduced with permission of Royal Society of Chemistry.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