

Electrostatics in molecular recognition: From ion pairs and inophores to nucleotides and DNA¹

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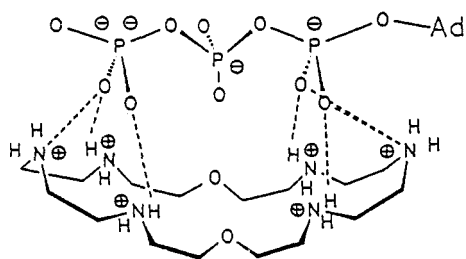
Abstract : The practical use as well as the empirical evaluation of electrostatic contributions in supramolecular complexes is reviewed on the basis of organic ion pairs, including amino-cyclodextrin - phosphate complexes and interactions between polyamines and DNA. The systematical analysis of these systems in water yield a surprisingly uniform value of 5 ± 1 kJ per Mol and salt bridge. Additional contributions from hydrogen bond and Van der Waals effects with the amino- cyclodextrins lead for nucleotides to simultaneous discrimination between nucleobases, 3'-/5'- phosphates, and 2'-oxy/deoxy derivatives. Electrostatic interactions in crown ether and cryptand complexes can be scaled by corresponding electron donor numbers for the different ligand atoms; the use of additive increments leads to a comprehensive description of corresponding alkali and ammonium ion complex stabilities.

The major role of electrostatic interactions in molecular recognition has been emphasized in many theoretical contributions aiming mostly at biopolymers². In the present short review we want to illustrate how Coulomb forces provide an essential basis for binding, particularly of nucleotides in synthetic host systems, and how the principle of additive pairwise interactions can be used to understand as well as to design supramolecular complexes based on these most important non-covalent forces. It is hoped, that the systematical analysis of synthetic host-guest complexes³, which in contrast to natural systems can be designed for the study of singular interactions in a often conformational well defined environment, also helps to obtain a firm basis for the rationalization of binding effects in proteins and nucleic acids as well as for the development of suitable effectors or drugs.

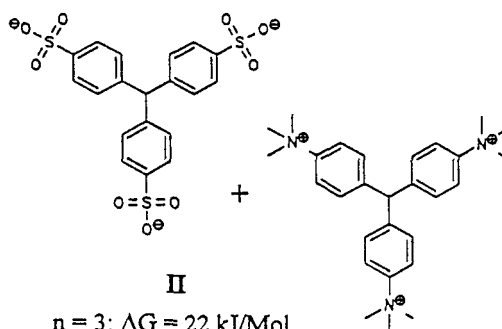
The formation of multiple salt bridges between e.g. nucleotide (phosphate) anions and protonated nitrogen atoms in azacrown ethers has been employed early by Kimura et al⁴ as well as by Lehn et al⁵ for the recognition of such phosphates in water. By comprehensive analyses of many ion pairs including ATP receptors such as I⁵ we have demonstrated that the stabilities of

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such complexes in water correlate linearly with the number of salt bridges between anion and cation ⁶, as long as there is sufficient match between the ionic sites, and any ligand strain induced by complexation is small compared to the gain of complexation energy ⁷. From over 60 ion pairs one obtains an increment of 5 ± 1 kJ/mol per salt bridge, which is surprisingly constant in view of the varying hard- and softness, as well as size of the participating ions, comprising, e.g., alkylated as well as protonated nitrogen, sulfonium and metal ions, or carboxylates, sulfonates, phosphates, halides, charge-delocalized phenolates etc. For some typical examples of such ion pairs shown below we give always the experimental total free complexation energy ΔG reported, the number n of salt bridges taken e.g. from suitable computer simulated models if necessary ⁷, and the resulting increment $\Delta\Delta G$. Aromatic ion pairs such as **II** display additional binding energies of usually ~ 2 kJ per Mol and ion-arene interaction ^{6b}; a similar increment for related Van der Waals contributions is observed in associations of ionic species and electroneutral arenes in water ⁸.

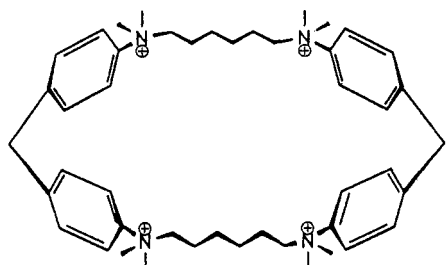


I (Lehn *et al.*)
 $n = 11 \pm 1$; $\Delta G = 63$ kJ/Mol
 $\Delta\Delta G = 5.5$ kJ/mol



II
 $n = 3$; $\Delta G = 22$ kJ/Mol
 (addtl. VdW interactions)

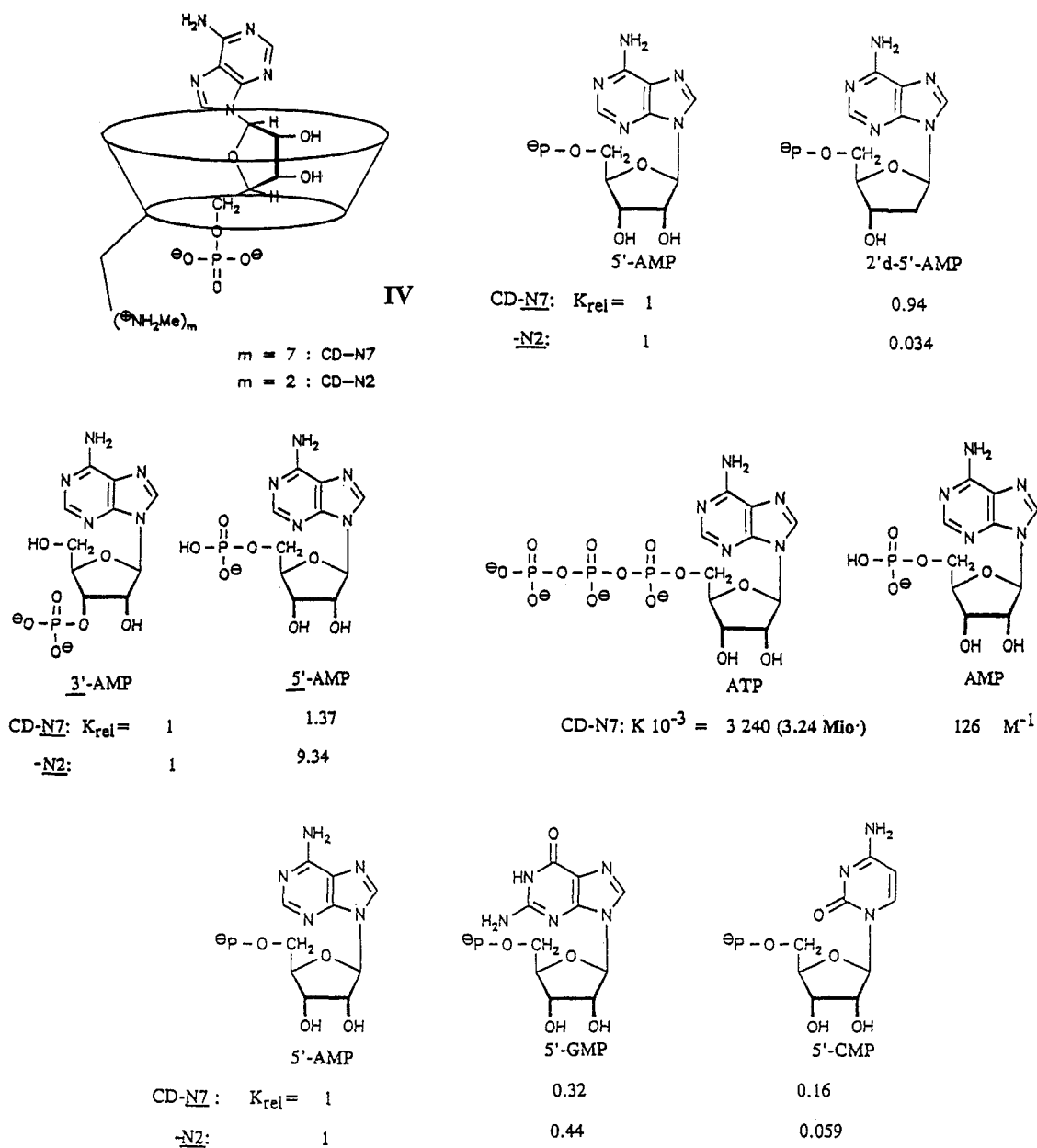
As long as the complexation of nucleotides is based solely on salt bridges the host can quite effectively differentiate between anion charges such as AMP, ADP, and ATP, but not between other features. Base recognition can be achieved by providing the host with additional "stacking" elements ^{9,10}, or by the use of host cavities suitable for encapsulation of the lipophilic parts within a cavity such as in CPnn **III** ¹¹. Although in the latter Van der Waals forces may actually dominate the remaining salt bridges can again be rationalized with the increment of 5 ± 1 kJ/mol.



III
 with R= Ribose 10 [kJ/Mol]
 R= Ribose-OPO₂²⁻ 19.3
 $\Delta\Delta G$ (Coulomb) 9.3 ; $n = 2$

Any host for nucleotides which is as efficient as natural receptors and which should be useful e.g. for sensor applications should be able to differentiate not only between the charges and the bases involved, but also between the site of the phosphate groups and between the presence or absence of hydroxy groups in the sugars. Recently we found that 6-desoxy-6-methylamino- β -cyclodextrins **IV**, bearing either 7 or 2 positively charged $^+\text{NH}_2\text{Me}$ groups (**CD-N7** or **CD-N2**) can in the *natural environment water* - fulfill *all* these functions including the distinction between 3'-or 5'-phosphates and 2'-oxy- or deoxy-ribose¹².

While ion pairing still provides the primary binding force in these systems, other factors such as hydrogen bonds between the cyclodextrin and the nucleobases are responsible for the high selectivity. Both molecular mechanics simulations as well as NMR measurements demonstrate



that it is indeed the sugar part which in spite of its hydrophilicity resides inside the hydrophobic CD cavity, in consequence with the nucleobase at the upper rim of the cycloamylose. Thus, only the sugar protons show large intermolecular NOEs upon irradiation at the protons *inside* the CD cavity, which are also the only ones showing appreciable complexation shifts. It is noteworthy, that the hydrogen bond and hydrophobic interactions, while important for the selectivity, do *not* contribute significantly to the overall binding ΔG . In fact they are for most nucleosides rather repulsive than attractive, as visible in a systematic comparison of complexation stabilities observed with inorganic phosphate, sugar phosphate alone, nucleoside, and nucleotide. In line with this, the selectivity *decreases* if one uses a heptaamino-CD with 7 charges (IV, $m=7$) instead a diamino-CD (IV, $m=2$) with only 2 positive charges. Thus, Coulomb forces alone lead to strong accumulative binding, which, however, can actually diminish the contribution of other forces responsible for fine-tuned molecular recognition.

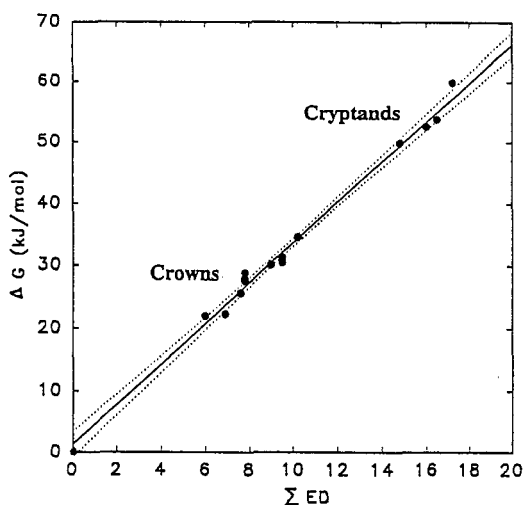


Figure 1:
Free energies of complexation ΔG
of selected ionophores with K^+ vs.
 ΣED (see text).

Coulomb interactions represent strong, but particularly "liberal" forces as they fall off only with r^{-1} between the charges. This we believe to be the reason for the recently found¹³ simple linear correlation of crown ether and cryptand stabilities with alkali and ammonium cations and the number n of ligand atoms provided by the ionophore, which offer the negative partial charge of oxygen or nitrogen lone pairs for cation binding (Figure 1). Here we scale the electron donor capacity ED of the different ligand atoms by independent measurements of 1:1 hydrogen bond associations with single ligand atoms in carbon tetrachloride¹⁴. Similarly, we observe a linear correlation between the cation complex stabilities and their relative hydration enthalpy¹³. Independent of any mechanistic reasoning, this incremental approach provides for the first time a simple and comprehensive description as well as prediction of such complex stabilities and the necessary geometric and chemical requirements for selectivity. It must be emphasized, however, that the LFER-type of analysis at the present state is limited to systems with sufficient contact between separated binding sites and negligible strain variations.

The binding of cationic substrates such as protonated polyamines¹⁵ to double-stranded DNA represents the biologically and pharmaceutically most important electrostatic interaction in nature (Figure 2, Scheme 1). Based on earlier equilibrium measurements¹⁶ and computer mo-

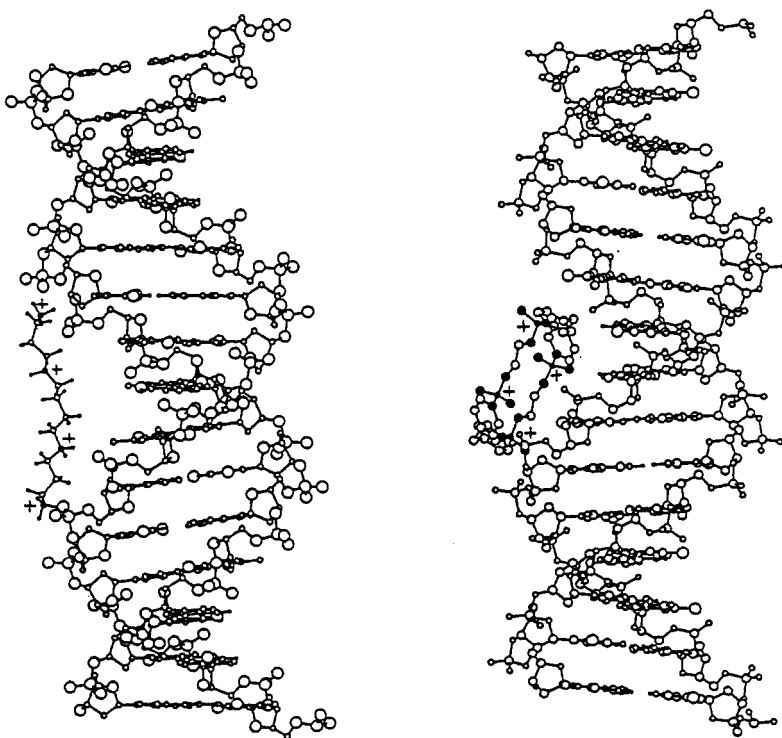
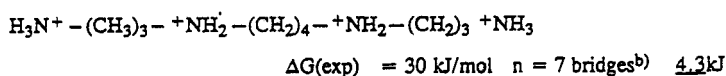
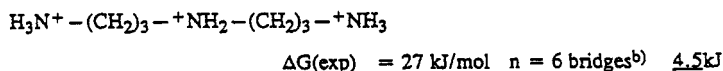
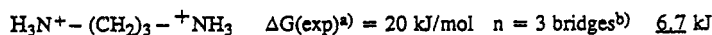


Figure 2. Polyamine binding to the major groove of ds-(B)-DNA; (a) of spermine; (b) of azoniacyclophane CP44 (similar to compound III, but with only 4 CH₂ between ⁺N atoms). The localisation of the positive charges is indicated.



Peralkylated systems (-(CH₂)_n-⁺NMe₂-(CH₂)_m-...) DNA Affinities:

Again: R - (⁺NMe₂)_m - R' are described with the 5 kJ increment.

Even with R = Aryl no addtl. intercalation (¹H-NMR: $\Delta\nu < 0.1$ ppm...; and viscosity measurements...)

Scheme 1. Some representative values for the affinity of polyamines to ds-(B)DNA.

delling studies¹⁷ identifying the number of possible salt bridges it can be shown that the affinity of both natural as well as synthetic polyamines¹⁸ to DNA again is ruled by the increment of ~5 kJ/Mol¹⁹. Macrocyclic tetramines like CPnn III usually show similar affinities as open chain analogs, although one which seems to fit particularly well into the major ds-DNA groove (Figure 2b) exhibits a somewhat larger value. The presence of aryl units in either open or cyclic polyamines does not lead to any intercalation as evident from NMR and viscosity measurements¹⁹. That it is indeed the Coulomb interaction and not, as often assumed, a hydrogen

bond contribution from the protonated amine which dominates the groove binding was established by observing the *same* affinities of *alkylated* polyamines with the same number of positive charges¹⁹. This is in accord with the observation of very similar association constants between cationic guest molecules and either protonated or peralkylated cyclophanes CP_n **III** as host.

REFERENCES

- 1) Supramolecular Chemistry, Part 42; part 41: Schneider, H.-J.; Kramer, R.; Rammo, J. *J. Am. Chem. Soc.* **1993**, *115*, in print.
- 2) Warshel, A.; Russell, S. T. *Quart. Rev. Biophys.* **1984**, *17*, 283.
- 3) Schneider, H.-J. *Angew. Chem.* **1991**, *103*, 1419; *Angew. Chem. Int. Ed. Engl.* **1990**, *30*, 1417.
- 4) Kimura, E. in: "Crown Ethers and Analogous Compounds"; Hiraoka, M., Ed., *Studies in Organic Chemistry* **45**; Tokyo **1992**, p. 381; Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182.
- 5) Lehn, J.-M., *Angew. Chem.* **1988**, *100*, 91; *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89, and earlier references.
- 6) a) Schneider, H.-J.; Theis, I. *Angew. Chem.* **1989**, *101*, 757; *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 753; 6b) Schneider, H.-J.; Schiestel, T.; Zimmermann, P. *J. Am. Chem. Soc.* **1992**, *114*, 7698.
- 7) See also Schneider, H.-J. *Rec. Trav. Chim. Pays-Bas* **1993**, *112*, 412.
- 8) Schneider, H.-J.; Werner, F.; Blatter, T.; *J. Phys. Org. Chem.* **1993**, *6*, in print.
- 9) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1988**, 596.
- 10) Rebek, J. Jr. *Angew. Chem.* **1990**, *102*, 261; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 245, and references cited therein.
- 11) Schneider, H.-J.; Blatter, T.; Palm, B.; Pfingst, U.; Rüdiger, V.; Theis, I. *J. Am. Chem. Soc.* **1992**, *114*, 7704.
- 12) Eliseev, A. V.; Schneider, H.-J. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, in print.
- 13) Schneider, H.-J.; Rüdiger, V.; Raevsky, O. A. *J. Org. Chem.* **1993**, *58*, 3648.
- 14) Iogansen, A. V. *Dokl. Acad. Nauk SSSR* **1965**, *164*, 610; *Chem. Abstr.* **1966**, *64*, 2868h, and ref. cited in 13); Raevsky, O. A. *Russ. Chem. Rev., Engl. Ed.* **1990**, *59*, 219.
- 15) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749.
- 16) Braunlin, W. H.; Strick, T. J.; Record, M. T. *Biopolymers* **1982**, *21*, 1301.
- 17) Stekowski, L.; Harden, D. B.; Wydra, R. L.; Stewart, K. D.; Wilson, W. D. *J. Mol. Recognit.* **1989**, *2*, 158.
- 18) See also Stewart, K. D.; Gray, T. A. *J. Phys. Org. Chem.* **1992**, *5*, 461; and references cited therein.
- 19) Schneider, H.-J.; Blatter, T. *Angew. Chem.* **1992**, *104*, 1244; *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1207.