Large Extra-cavity Contributions to Hydrophobic Binding of Lipophilic Substrates in Synthetic Receptor Models

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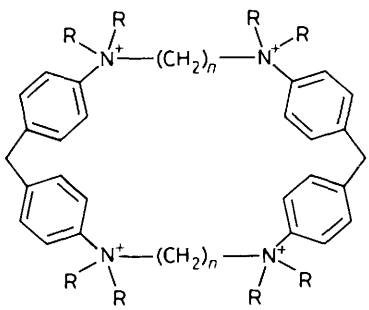
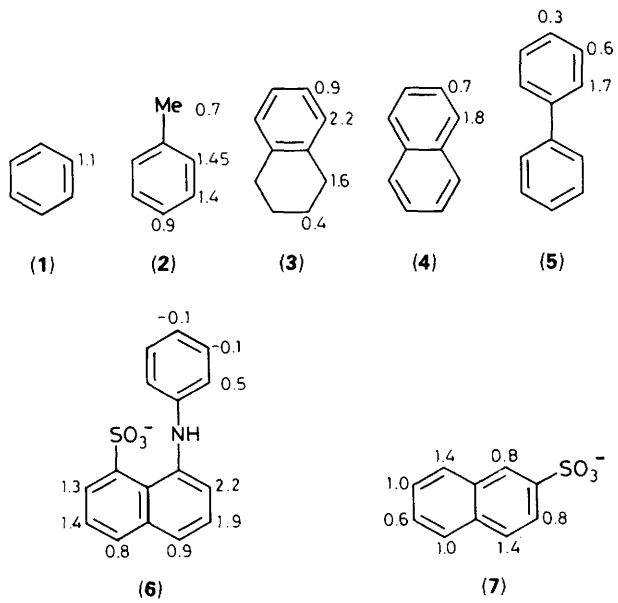
On the basis of equilibrium constants with macrocyclic azonia cyclophanes, of complexation induced NMR shifts, and of computer simulated structures, it is shown that lipophilic guest parts extending out of the host cavity greatly enhance the binding in water.

Ever since Emil Fischer coined the lock–key picture for enzyme substrate complexes, the interaction between structural elements of biopolymer cavities and smaller substrates has been the focus of many studies involving interactions with receptor proteins, nucleic acids, drugs *etc.* In the traditional approach, the non-covalent interactions between those receptor and substrate elements which are in proximity are analysed in order to evaluate strength and specificity of the binding; recent applications involve the development of new proteins by site-directed mutagenis. We want to communicate experimental evidence suggesting that structural parts outside the cavity greatly influence the formation constants of such complexes in water.



CP66 : n = 6; R = Me
CP44 : n = 4; R = H

Synthetic receptor analogues of the type *CPnn* have been shown to bind lipophilic substrates such as (**1**)—(**7**) (Table 1) quite effectively. Although their relatively open cavity is only lined by phenyl groups and methylene chains the observed binding constants[1,2] in water *e.g.*, with the fluorescence dye



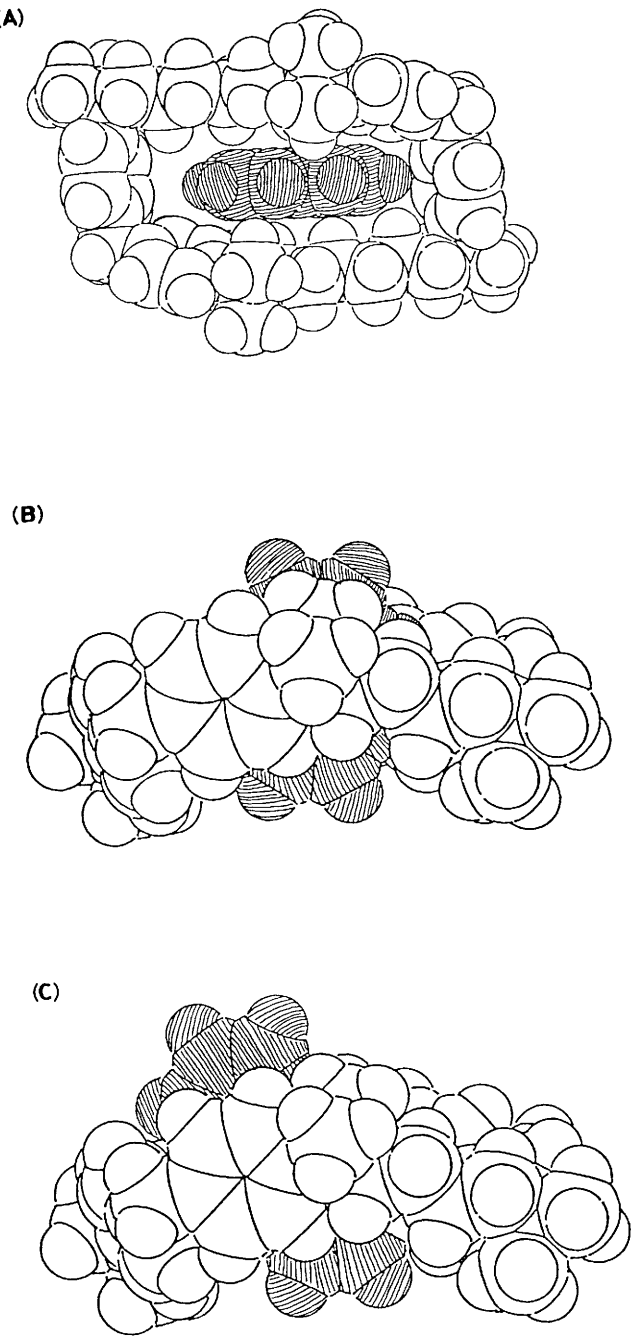


Figure 1. Force field simulated structures (CHARMm, QUANTA) for structures, (**A**): top view of naphthalene (**4**) with pseudoequatorial inclusion in *CP66*. (**B**): side view of naphthalene (**4**) in *CP44*. (**C**): side view of biphenyl (**5**) in *CP44*. The substrate parts extending out of the cavity are shaded.

1-anilinonaphthyl-8-sulphonate (ANS) (**6**) amount to 4.10^5 mol^{+1} dm^3. The inclusion within the cavity is visible in the complexation induced ^{1}H NMR shifts, which are obtained from a suitable computer fit of NMR spectroscopic titrations.[3] The titrations were carried out by adding *CPnn* host stock solutions in usually eight increments to solutions of the guest in D_2O (typical concentrations, 3.10^{-4}—8.10^{-3} M, depending on the constant and solubility), aiming at values between 20 and 80% complexation. The shift of all non-equivalent protons of the guest molecule were followed as far as possible, yielding equilibrium constants, K, which usually differed by

Table 1. Substrates (**1**)—(**7**) complexed with *CP44* or *CP66*. Free energies of complexation ΔG° and complexation induced NMR shifts (CIS in p.p.m.) for the observed protons; measurements in D_2O at 300 ± 2 K.

	(**1**)[a]	(**2**)[a]	(**3**)[a]	(**4**)[a]	(**5**)[a]	(**6**)[b]	(**7**)[b]
$-\Delta G^\circ$/kJ mol^{-1}	17.1	17.2	18.4	20.9	22.3	32.0	23.3
$K_A \times 10^{-3}$/mol^{-1} dm^3	0.99	1.03	1.70	4.49	8.07	400	12.1

[a] With *CP44*. [b] With *CP66*.

<5%, and NMR complexation shifts with an accuracy of ±0.03 p.p.m.

The shifts obtained from the computer fit for 100% complexation [complexation induced NMR shift (CIS) values, indicated in Table 1] prove the immersion of the substrates in the cavity on the basis of aromatic ring anisotropy and electrical field effect calculations.[4] The dominant hydrophobic or lipophilic nature of the responsible interactions is also obvious, in the case of additional electrostatic attractions [substrates (**6**) and (**7**)], from comparative solvent hydrophobicity effects.[3] An important contribution to the binding of aromatic substrates is the special interaction of the positively charged nitrogen atoms with the π-systems of the substrates.[5—7]

Computer simulations with the CHARMm force field[8] (see Figure 1) demonstrate in line with results obtained earlier with the Biosym field,[9] that the larger cyclophane *CP66* can accommodate a naphthalene moiety [Figure 1 (**A**)] with a pseudoequatorial orientation; this is supported by the observed NMR–CIS values.[4]

In contrast, the smaller host compound *CP44* is tailor-made to surround naphthalene and similar substrates only in the axial or nearly axial position, which leads to a substantial exposure of substrate parts out of the cavity [Figure 1 (**B**) and (**C**)]. If we now enlarge these extra-cavity parts systematically from substrate (**1**) to (**5**) we observe a substantial and regular increase in binding (Table 1). The observed small complexation NMR shifts *e.g.*, in the *para* position of the biphenyl (**5**), as well as on the phenyl ring in (**6**) (ANS), demonstrate that these substrate parts are remote from the cavity. These parts, however, lead to a large increase of association constants, in the case of (**6**) *e.g.*, by a factor of 33.

It should be noted that both the observed NMR shifts as well as the perfect fit, obtained in the shift titrations on the basis of a 1 : 1 complex model, eliminate significant contributions of 1 : 2 complexes in which a second macrocycle surrounds the other part of the substrates. Such 1 : 2 complexes have been found *e.g.*, with cyclodextrins[10] which lack the charges leading to the destabilization of macrocycle association in the case of the azoniacyclophanes *CP44* or *CP66*. [The possibility of 1 : 2 complex formation besides the solubility problem makes it difficult to use larger lipophilic substrates than (**5**) for the demonstration of the extra-cavity effect.]

The results demonstrate for the first time that substrate parts outside the cavity increase the binding constants by factors up to powers of magnitude.[11] The consequence of the observed positive extra-cavity contributions to binding is that the traditional interaction analysis on the basis of *X*-ray determined structures, of NMR studies, of (computer aided) molecular modelling, or of modified proteins must be expanded correspondingly. The use of free energy pertubation methods[12—15] holds particular promise as it takes into account solvation shells of substrates and receptors which obviously extend significantly beyond the cavity.

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