

ITC XX Cyclodextrin-Guest Molecule Interactions

Auletta T., de Jong M. R., Mulder A., van Veggel F. C., Huskens J., Reinhoudt D. N., Zou S., Zapotoczny S., Schonherr H., Vancso G. J., and Kuipers L. (2004) Beta-cyclodextrin host-guest complexes probed under thermodynamic equilibrium: thermodynamics and AFM force spectroscopy. *J Am Chem Soc* **126**, 1577-1584.

Abstract: The rupture forces of individual host-guest complexes between beta-cyclodextrin (beta-CD) heptathioether monolayers on Au¹¹¹ and several surface-confined guests were measured in aqueous medium by single molecule force spectroscopy using an atomic force microscope. Anilyl, toluidyl, tert-butylphenyl, and adamantylthiols (0.2-1%) were immobilized in mixed monolayers with 2-mercaptoethanol on gold-coated AFM tips. For all guests and for all surface coverages, the force-displacement curves measured between the functionalized tips and monolayers of beta-CD exhibited single, as well as multiple, pull-off events. The histograms of the pull-off forces showed several maxima at equidistant forces, with force quanta characteristic for each guest of 39 +/- 15, 45 +/- 15, 89 +/- 15, and 102 +/- 15 pN, respectively. These force quanta were independent of the loading rate, indicating that, because of the fast complexation/decomplexation kinetics, the rupture forces were probed under thermodynamic equilibrium. The force values followed the same trend as the free binding energy ΔG degrees measured for model guest compounds in solution or on beta-CD monolayers, as determined by microcalorimetry and surface plasmon resonance measurements, respectively. A descriptive model was developed to correlate quantitatively the pull-off force values with the ΔG° of the complexes, based on the evaluation of the energy potential landscape of tip-surface interaction.

Bistri O., Mazeau K., uzely-Velty R. and Sollogoub M. (2007) A hydrophilic cyclodextrin duplex forming supramolecular assemblies by physical cross-linking of a biopolymer. *Chemistry* **13**, 8847-8857.

Abstract: New beta-cyclodextrin (beta-CD) dimeric species have been synthesised in which the two CD moieties are connected by one or two hydrophilic oligo(ethylene oxide) spacers. Their complexation with sodium adamantylacetate (free adamantane) and adamantane-grafted chitosan (AD-chitosan) was then studied by different complementary techniques and compared with their hydrophobic counterparts that contain an octamethylene spacer. Isothermal titration calorimetry experiments have demonstrated that the use of hydrophilic spacers between the two CDs instead of aliphatic chains makes almost all of the CD cavities available for the inclusion of free adamantane. Investigation of the interaction of the CDs with AD-chitosan by viscosity measurements strongly suggests that the molecular conformation of the CD dimeric species plays a crucial role in their cross-linking with the biopolymer. The derivative doubly linked with hydrophilic arms, also called a duplex, has been shown to be a more efficient cross-linking agent than its singly bridged counterpart, referred to as a dimer. Hence, only 0.5 molar equivalents of the hydrophilic duplex with respect to adamantane was required to obtain the maximum viscosity, whereas in the case of the duplex with aliphatic spacers, the maximum viscosity was achieved with a [duplex]/[AD] ratio of about 1.7 (corresponding to a [CD]/[AD] ratio of 2.5), but with a higher value. To clarify the relationships between the molecular architecture and complexation properties, computational studies were also performed that clearly confirmed the importance of double bridging.

Bouchemal K. (2008) New challenges for pharmaceutical formulations and drug delivery systems characterization using isothermal titration calorimetry. *Drug Discov. Today* **13**, 960-972.

Abstract: Long viewed as the 'method of choice' for characterizing thermodynamics and stoichiometry of molecular interactions, with high sensitivity, isothermal titration calorimetry (ITC) has been applied to many areas of pharmaceutical analysis. This review highlights ITC employment to measure binding thermodynamics and their use for pharmaceutical formulations and drug delivery system characterization particularly cyclodextrin-guest interactions, investigation of micellar-based systems, polyelectrolytes, nucleic acid interactions with multivalent cations and the optimization of DNA targeting and delivery. Furthermore, the potential of ITC for the characterization of different functionalities carried by nanoparticles as well as their interaction with living systems was outlined

Castronuovo G. and Niccoli M. (2006) Thermodynamics of inclusion complexes of natural and modified cyclodextrins with propranolol in aqueous solution at 298 K. *Bioorg Med Chem* **14**, 3883-3887.

Abstract: The association constant, standard Gibbs energy, enthalpy and entropy for formation of inclusion

complexes of propranolol, a beta-blocker, with various natural and modified cyclodextrins have been determined by calorimetry at 298 K. Both natural and methyl-modified alpha-cyclodextrins do not form complexes, while beta- and gamma-cyclodextrins do. Complexing ability of 2-hydroxypropyl-beta-cyclodextrin depends on the average substitution degree. For gamma-cyclodextrin, hydrophobic interactions play the major role in binding the guest. The association of natural and modified beta-cyclodextrins is ruled by van der Waals interactions and hydrogen bonding because of the tighter fit of the guest into the cavity. Decreasing pH determines increasingly negative values of the association enthalpies.

Charlot A., Heyraud A., Guenot P., Rinaudo M., and Auzely-Velty R. (2006) Controlled synthesis and inclusion ability of a hyaluronic acid derivative bearing beta-cyclodextrin molecules. *Biomacromolecules* **7**, 907-913.

Abstract: A new synthetic route to beta-cyclodextrin-linked hyaluronic acid (HA-CD) was developed. This was based on the preparation of a HA derivative selectively modified with adipic dihydrazide (HA-ADH) and a beta-cyclodextrin derivative possessing an aldehyde function on the primary face, followed by their coupling by a reductive amination-type reaction. The CD-polysaccharide was fully characterized in terms of chemical integrity and purity by high-resolution NMR spectroscopy. The complexation ability of the grafted CD was further demonstrated by isothermal titration calorimetry using sodium adamantane acetate (ADAc) and Ibuprofen as model guest molecules. The thermodynamic parameters for the complexation of these negatively charged guest molecules by the beta-CD grafted on negatively charged HA were shown to be largely influenced by the ionic strength of the aqueous medium.

Charman S. A., Perry C. S., Chiu F. C., McIntosh K. A., Prankerd R. J., and Charman W. N. (2006) Alteration of the intravenous pharmacokinetics of a synthetic ozonide antimalarial in the presence of a modified cyclodextrin. *J Pharm Sci* **95**, 256-267.

Abstract: The pharmacokinetic profile and renal clearance of a novel synthetic ozonide antimalarial (1) was found to be significantly altered when intravenously administered to rats as a cyclodextrin-based formulation (0.1 M Captisol, a sulfobutylether beta-cyclodextrin derivative (SBE(7)-beta-CD)) compared to a cyclodextrin-free isotonic buffered glucose formulation. There was an 8.5-fold decrease in the steady-state blood volume of distribution, a 6.6-fold decrease in the mean residence time and a greater than 200-fold increase in renal clearance of 1 when administered in the cyclodextrin formulation. Analysis of the whole blood and plasma concentration profiles revealed an essentially constant blood to plasma ratio when 1 was administered in the cyclodextrin-free formulation, whereas this ratio changed as a function of time when administered in the presence of the cyclodextrin derivative. It is postulated that the observed differences were due to a very strong complexation interaction between 1 and the cyclodextrin, resulting in a slow dissociation of the complex in vivo, and altered distribution and excretion profiles. Preliminary studies using isothermal titration calorimetry (ITC) indicated that the association constant for the 1/Captisol complex was approximately two orders of magnitude higher than reported for typical drug/cyclodextrin complexes.

Cooper A., Nutley M., Maclean E. J., Cameron K., Fielding L., Mestres J., and Palin R. (2005) Mutual induced fit in cyclodextrin-rocuronium complexes. *Org Biomol Chem* **3**, 1863-1871.

Abstract: The binding of rocuronium bromide to 6-perdeoxy-6-per(4-carboxyphenyl)thio-[gamma]-cyclodextrin sodium salt, displays biphasic behaviour characteristic of the formation of a binary and 2 : 1 ternary guest-host complex in aqueous solution. Thermodynamic and structural data on this sequential complexation process can be rationalised within a single model involving switching of the conformational equilibria of both the rocuronium bromide and cyclodextrin molecules. Isothermal titration calorimetry (ITC), NMR and fluorescence experiments in solution, together with X-ray crystallography and molecular modelling, suggest that in order to induce encapsulation both rocuronium bromide and the modified cyclodextrin undergo conformational changes. Ring A of rocuronium bromide 'switches' from the more sterically encumbered chair to the sterically less demanding twist-boat, whilst the modified cyclodextrin "opens" its cavity to allow the steroid to enter. The recognition and mutual induced fit between cyclodextrin and steroid represents a classic example of dynamic host-guest chemistry.

Denadai A. M., Santoro M. M., Lopes M. T., Chenna A., de Sousa F. B., Avelar G. M., Gomes M. R., Guzman F., Salas C. E., and Sinisterra R. D. (2006) A supramolecular complex between proteinases and beta-cyclodextrin that preserves enzymatic activity: physicochemical characterization. *BioDrugs* **20**, 283-

Abstract: BACKGROUND: Cyclodextrins are suitable drug delivery systems because of their ability to subtly modify the physical, chemical, and biological properties of guest molecules through labile interactions by formation of inclusion and/or association complexes. Plant cysteine proteinases from Caricaceae and Bromeliaceae are the subject of therapeutic interest, because of their anti-inflammatory, antitumoral, immunogenic, and wound-healing properties. METHODS: In this study, we analyzed the association between beta-cyclodextrin (betaCD) and fraction P1G10 containing the bioactive proteinases from *Carica candamarcensis*, and described the physicochemical nature of the solid-state self-assembled complexes by Fourier transform infrared (FTIR) spectroscopy, thermogravimetry (TG), differential scanning calorimetry (DSC), X-ray powder diffraction (XRD), and nuclear magnetic resonance (NMR), as well as in solution by circular dichroism (CD), isothermal titration calorimetry (ITC), and amidase activity. RESULTS AND DISCUSSION: The physicochemical analyses suggest the formation of a complex between P1G10 and betaCD. Higher secondary interactions, namely hydrophobic interactions, hydrogen bonding and van der Waals forces were observed at higher P1G10 : betaCD mass ratios. These results provide evidence of the occurrence of strong solid-state supramolecular non-covalent interactions between P1G10 and betaCD. Microcalorimetric analysis demonstrates that complexation results in a favorable enthalpic contribution, as has already been described during formation of similar betaCD inclusion compounds. The amidase activity of the complex shows that the enzyme activity is not readily available at 24 hours after dissolution of the complex in aqueous buffer; the proteinase becomes biologically active by the second day and remains stable until day 16, when a gradual decrease occurs, with basal activity attained by day 29. CONCLUSION: The reported results underscore the potential for betaCDs as candidates for complexing cysteine proteinases, resulting in supramolecular arrays with sustained proteolytic activity.

Denadai A. M., Ianzer D., Alcantara A. F., Santoro M. M., Santos C. F., Lula I. S., de Camargo A. C., Faljoni-Alario A., dos Santos R. A. and Sinisterra R. D. (2007) Novel pharmaceutical composition of bradykinin potentiating penta peptide with beta-cyclodextrin: physical-chemical characterization and anti-hypertensive evaluation. *Int J Pharm.* **336**, 90-98.

Abstract: This work describes chemical properties and anti-hypertensive activity of an oral pharmaceutical formulation obtained from the complexation of beta-cyclodextrin (beta-CD) with bradykinin potentiating penta peptide (BPP-5a) founded in the Bothrops jararaca poison. Physical chemistry characterizations were recorded in order to investigate the intermolecular interactions between species in complex. Circular dichroism data indicated conformational changes of BPP-5a upon complexation with beta-CD. ROESY and theoretical calculations showed a selective approximation of triptophan moiety into cavity of beta-CD. Isothermal titration calorimetry data indicated an exothermic formation of the complex, which is accomplished by reduction of entropy. The anti-hypertensive activity of the BPP-5a/beta-CD complex has been evaluated in spontaneous hypertensive rats, showing better results than pure BPP-5a.

Denadai A. M., Teixeira K. I., Santoro M. M., Pimenta A. M., Cortes M. E. and Sinisterra R. D. (2007) Supramolecular self-assembly of beta-cyclodextrin: an effective carrier of the antimicrobial agent chlorhexidine. *Carbohydr. Res* **342**, 2286-2296.

Abstract: The supramolecular assembly between chlorhexidine and cyclomaltoheptaose (beta-cyclodextrin, betaCD) was characterized using NMR spectroscopy (¹H, T(1), and ROESY), ESIMS and ITC. NMR data suggest the formation of high ordered complexes. ESIMS and ITC allowed the confirmation of the average stoichiometry as 1:4 and the thermodynamic data, also obtained by ITC, showed that the assembly is strongly stabilized by short distance interactions, but suffers a strong, opposite effect of entropy reduction. The antimicrobial activity of 1:1, 1:2, 1:3, and 1:4 Clx/betaCD molar ratio mixtures was investigated in aqueous solution and after incorporation into mucoadhesive gels. These were used to determine the initial and the long-term antimicrobial activity, respectively, toward *Actinobacillus actinomycetemcomitans* (A.a.) (Y4-FDC) and *Enterococcus faecalis* (E.f.) (ATCC 14508) strains. The results showed that A.a. and E.f. were more susceptible to the 1:4 molar ratio mixture in either solution or gel (p<0.05).

De Sousa F. B., Denadai A. M., Lula I. S., Nascimento C. S., Jr., Fernandes Neto N. S., Lima A. C., De Almeida W. B. and Sinisterra R. D. (2008) Supramolecular self-assembly of cyclodextrin and higher water soluble guest: thermodynamics and topological studies. *J Am. Chem Soc.* **130**, 8426-8436.

Abstract: The supramolecular interactions between Imipramine hydrochloride (IMI), a tricyclic antidepressant, and beta-cyclodextrin (betaCD) have been investigated by experimental techniques and theoretical calculations. The association between these molecules might lead to a host/guest compound, in which the physical chemistry properties of the guest molecule, such as high solubility, can be decreased. These new properties acquired by the inclusion phenomena are important to develop a strategy for pharmaceutical formulation. Nuclear magnetic resonance and horizontal attenuated total reflectance provided relevant information on the complex stoichiometries and the sites of interactions between the host and guest molecules. Stoichiometries of 1:2, 1:1, and 2:1 betaCD/IMI have been detected in solution. Self-diffusion coefficient and dynamic light scattering analysis provided information on the self-aggregation of the complex. Also, isothermal titration calorimetry studies indicated the existence of equilibrium between different complexes in solution. In order to determine the preferred arrangement for the inclusion complex formed by the IMI molecule and betaCD, theoretical calculations were performed. Of all proposed supramolecular structures, the 2:1 betaCD/IMI complex was calculated to be the most energetically favorable, in both gas and aqueous phases. The calculations indicated that the intermolecular hydrogen bonds involving the hydroxyl groups of betaCD play a major role in stabilizing the supramolecular 2:1 structure, corroborating experimental findings

De Sousa F. B., Denadai A. M., Lula I. S., Lopes J. F., Dos Santos H. F., De Almeida W. B. and Sinisterra R. D. (2008) Supramolecular complex of fluoxetine with beta-cyclodextrin: an experimental and theoretical study. *Int J Pharm.* **353**, 160-169.

Abstract: In this work the complex formed between beta-cyclodextrin (betaCD) and fluoxetine (FLU) was investigated by experimental and computational methods. From Horizontal Attenuated Total Reflectance (HATR) was possible to verify a strong modification in the vibrational modes of betaCD and FLU, indicating interactions between them. The Nuclear Magnetic Resonance (NMR) experiments confirm these interactions through the change in chemical shifts in ^1H spectra, reduction in longitudinal relaxation times values, and the Nuclear Overhauser Effect confirm the inclusion of aromatic rings of FLU into the betaCD. The structures of the proposed inclusion compounds were optimized at PM3 semiempirical level of theory. In addition, single point calculations at the Density Functional Theory (DFT) level, using the Becke, Lee, Yang, and Parr functional and 6-31G(d,p) basis set, were used to determine the interaction energy for these structures. The DFT calculations identified the aromatic ring, which contains the CF(3) group as the most stable into the betaCD by an amount of, 11.7 kcal mol⁻¹, in the gas phase. Polarized continuum model, at the DFT level mentioned, was used to investigate the solvent effect, and the results corroborated the gas phase analysis. A high equilibrium constant (K approximately 6921 \pm 316) and the stoichiometry, 1:1, were obtained by Isothermal Titration Calorimetry (ITC) experiments

Frycak P. and Schug K. A. (2008) Dynamic titration: determination of dissociation constants for noncovalent complexes in multiplexed format using HPLC-ESI-MS. *Anal Chem* **80**, 1385-1393.

Abstract: With recent growth in fields such as life sciences and supramolecular chemistry, there has been an ever increasing need for high-throughput methods that would permit determination of binding affinities for noncovalent complexes of various host-guest systems. These are traditionally measured by titration experiments where concentration-dependent signals of species participating in solution-based binding equilibria are monitored by methods such as UV-vis spectrophotometry, calorimetry, or nuclear magnetic resonance spectrometry. Here we present a new titration technique that unifies and allows chromatographic separation of guests with determination of dissociation constants by electrospray mass spectrometry in a multiplexed format. A theoretical model has been derived that describes the complex formation for the guests eluted from a chromatographic column when hosts are admixed postcolumn. The model takes possible competition equilibria into account; i.e., it can deal with unresolved peaks of guests with the possible addition of multiple hosts in one experiment. This on-line workflow makes determination of binding affinities for large libraries of compounds possible. The potential of the method is demonstrated on the determination of dissociation constants for complexes of beta- and gamma-cyclodextrins with nonsteroidal antiinflammatory drugs ibuprofen, naproxen, and flurbiprofen

Gomez-Biagi R. F., Jagt R. B. and Nitz M. (2008) Remarkably stable inclusion complexes with heptakis-[6-deoxy-6-(2-aminoethylsulfanyl)]-beta-cyclodextrin. *Org. Biomol. Chem* **6**, 4622-4626.

Abstract: Complexes of heptakis-[6-deoxy-6-(2-aminoethylsulfanyl)]-beta-cyclodextrin () and a series of common cyclodextrin guests were studied by NMR, fluorescence spectroscopy, and ITC experiments.

NMR conformational analysis shows that the thioethers of are positioned over the hydrophobic cavity of the cyclodextrin, increasing potential hydrophobic interactions with guest molecules. The combination of the increased hydrophobic character, the electrostatic complementarity and a hypothesized conformational change in lead to a complex with the dye 2,6-ANS () that is over 2000 times more stable than with the native beta-cyclodextrin. One of the most stable host-guest complexes between a cyclodextrin and a small molecule measured to date was revealed between and lithocholic acid () with an association constant of $5.5 \times 10^7 \text{ M}^{-1}$

Guo M., Jiang M. and Zhang G. (2008) Surface modification of polymeric vesicles via host-guest inclusion complexation. *Langmuir* **24**, 10583-10586.

Abstract: We have prepared a novel kind of building block (CD-PI-CD) of polyether imide (PI) with beta-cyclodextrin (beta-CD) at the both ends that self-assembles into vesicles in water. The vesicles are "reactive" in supramolecular chemistry because the host groups of beta-CD equally distribute on the inside and outside surfaces of the vesicles. Isothermal titration calorimetry (ITC) and static light scattering (SLS) studies demonstrate that both the inner and outer surfaces of the vesicles can be further modified noncovalently with adamantane-ended polyethylene glycol (Ada-PEG) via a host-guest inclusion interaction between beta-CD and adamantane

Harries D., Rau D. C., and Parsegian V. A. (2005) Solutes probe hydration in specific association of cyclodextrin and adamantane. *J Am Chem Soc* **127**, 2184-2190.

Abstract: Using microcalorimetry, we follow changes in the association free energy of beta-cyclodextrin (CD) with the hydrophobic part of adamantane carboxylate (AD) due to added salt or polar (net-neutral) solutes that are excluded from the molecular interacting surfaces. Changes in binding constants with solution osmotic pressure (water activity) translate into changes in the preferential hydration upon complex formation. We find that these changes correspond to a release of 15-25 solute-excluding waters upon CD/AD association. Reflecting the preferential interaction of solute with reactants versus products, we find that changes in hydration depend on the type of solute used. All solutes used here result in a large change in the enthalpy of the CD-AD binding reaction. In one class of solutes, the corresponding entropy change is much smaller, while in the other class, the entropy change almost fully compensates the solute-specific enthalpy. For many of the solutes, the number of waters released correlates well with their effect on air-water surface tensions. We corroborate these results using vapor pressure osmometry to probe individually the hydration of reactants and products of association, and we discuss the possible interactions and forces between cosolute and hydrophobic surfaces responsible for different kinds of solute exclusion.

Hembury G., Rekharsky M., Nakamura A., and Inoue Y. (2000) Direct correlation between complex conformation and chiral discrimination upon inclusion of amino acid derivatives by beta- and gamma-cyclodextrins. *Org Lett* **2**, 3257-3260.

Abstract: A correlation between the conformation and chiral recognition characteristics of a series of modified amino acid complexes with beta- and gamma-cyclodextrins has been determined, using titration microcalorimetry and (1)H NMR techniques. The enantiomeric discrimination (D or L) is found to be dependent on the adoption of one of two distinct conformations. With the magnitude of the chiral discrimination (K(D)/K(L)) arising from the guest's depth of penetration into the host's cavity.

Hernandez-Pascacio J., Garza C., Banquy X., az-Vergara N., Amigo A., Ramos S., Castillo R., Costas M. and Pineiro A. (2007) Cyclodextrin-based self-assembled nanotubes at the water/air interface. *J Phys. Chem B* **111**, 12625-12630.

Abstract: Native alpha-cyclodextrin (alpha-CD) is found to spontaneously form films at aqueous solution/air interfaces. Shape-response measurements to volume perturbations on drops hanging from a capillary indicate that temperature and sodium dodecyl sulfate (SDS) concentration strongly modify the viscoelastic properties of such films. By using isothermal titration calorimetry (ITC), Brewster angle microscopy (BAM), atomic force microscopy (AFM), and molecular dynamics (MD) simulations, it is shown that the films consist of self-assembled nanotubes whose building blocks are cyclodextrin dimers (alpha-CD₂) and alpha-CD₂-SDS₁ complexes.

Ikeda H., Li Q., and Ueno A. (2006) Chiral recognition by fluorescent chemosensors based on N-dansyl-amino acid-modified cyclodextrins. *Bioorg Med Chem Lett* **16**, 5420-5423.

Abstract: Four kinds of N-dansyl-amino acid-modified beta-cyclodextrins (beta-CDs) were prepared as fluorescent chemosensors for chiral discrimination. The use of an amino acid as a spacer improved binding affinities and chiral discrimination abilities of the chemosensors. N-dansyl-L-Phe-modified beta-CD showed high d-selectivity for norbornane derivatives and N-dansyl-D-Phe-modified beta-CD showed high l-selectivity for menthol. Microcalorimetric titration results indicate that the chemosensors selectively accommodate the enantiomer that induces the least unfavorable entropy change on making an inclusion complex.

Illapakurthy A. C., Wyandt C. M., and Stodghill S. P. (2005) Isothermal titration calorimetry method for determination of cyclodextrin complexation thermodynamics between artemisinin and naproxen under varying environmental conditions. *Eur J Pharm Biopharm* **59**, 325-332.

Abstract: A novel isothermal titration calorimetry method was used to determine the complexation thermodynamics for hydroxypropyl-beta-cyclodextrin with artemisinin and naproxen at varying temperature and pH. The new method is very useful for studying complexation reactions between cyclodextrin and drugs with poor solubility and all the thermodynamic parameters of the cyclodextrin complexation were determined. The analysis of the thermodynamic data reveals involvement of hydrophobic bonding in the cyclodextrin complexes studied. The data also reveals the presence of enthalpy-entropy compensation in the system and provide information as to the orientation of the drug molecule inside the cyclodextrin cavity. From the thermodynamic parameters for dissociation of HPBCD complexes of artemisinin and naproxen at pH 2 it is concluded that the complexation is primarily driven by enthalpy with entropic assistance at all temperatures studied. From the dissociation studies of HPBCD complexes of naproxen at pH 10 it is concluded that the complexation is predominantly driven by entropy and moderately by enthalpy at lower temperatures and by enthalpy with entropic assistance at higher temperatures.

Izutani Y., Kanaori K., Imoto T., and Oda M. (2005) Interaction of gymnemic acid with cyclodextrins analyzed by isothermal titration calorimetry, NMR and dynamic light scattering. *FEBS J* **272**, 6154-6160.

Abstract: The physiological phenomenon that the antisweet taste effect of gymnemic acid (GA) is diminished by application of gamma-cyclodextrin (gamma-CD) to the mouth was evaluated at the molecular level using isothermal titration calorimetry, NMR and dynamic light scattering. These analyses showed that GA specifically binds to gamma-CD. Thermodynamic analysis using isothermal titration calorimetry revealed that the association constant of GA and gamma-CD is $10(5)$ - $10(6)$ m^{-1} with favorable enthalpy and entropy changes. The heat capacity change was negative and large, despite the change in accessible surface area upon binding being small. These thermodynamics indicate that the binding is dominated by hydrophobic interactions, which is in agreement with inclusion complex formation of gamma-CD. In addition, NMR measurements showed that in solution the spectra of GA are broad and sharpened by the addition of gamma-CD, indicating that unbound GA is in a water-soluble aggregate that is dispersed when it forms a complex with gamma-CD. Dynamic light scattering showed that the average diameter of unbound GA is > 30 nm and that of GA and gamma-CD complex is 2.2 nm, similar to unbound gamma-CD, supporting the aggregate property of GA and the inclusion complexation of GA by gamma-CD.

Jeon W. S., Moon K., Park S. H., Chun H., Ko Y. H., Lee J. Y., Lee E. S., Samal S., Selvapalam N., Rekharsky M. V., Sindelar V., Sobransingh D., Inoue Y., Kaifer A. E., and Kim K. (2005) Complexation of ferrocene derivatives by the cucurbit[7]uril host: a comparative study of the cucurbituril and cyclodextrin host families. *J Am Chem Soc* **127**, 12984-12989.

Abstract: The formation of inclusion complexes between cucurbit[7]uril (CB[7]) and ferrocene and its derivatives has been investigated. The X-ray crystal structure of the 1:1 inclusion complex between ferrocene and CB[7] revealed that the guest molecule resides in the host cavity with two different orientations. Inclusion of a set of five water-soluble ferrocene derivatives in CB[7] was investigated by 1H NMR spectroscopy and calorimetric and voltammetric techniques. Our data indicate that all neutral and cationic guests form highly stable inclusion complexes with CB[7], with binding constants in the $10(9)$ - $10(10)$ M^{-1} and $10(12)$ - $10(13)$ M^{-1} ranges, respectively. However, the anionic ferrocenecarboxylate, the only negatively charged guest among those surveyed, was not bound by CB[7] at all. These results are in sharp contrast to the known binding behavior of the same guests to beta-cyclodextrin (beta-CD), since all the guests form stable inclusion complexes with beta-CD, with binding constants in the range $10(3)$ - $10(4)$

M(-1). The electrostatic surface potentials of CB[6], CB[7], and CB[8] and their size-equivalent CDs were calculated and compared. The CD portals and cavities exhibit low surface potential values, whereas the regions around the carbonyl oxygens in CBs are significantly negative, which explains the strong affinity of CBs for positively charged guests and also provides a rationalization for the rejection of anionic guests. Taken together, our data suggest that cucurbiturils may form very stable complexes. However, the host-guest interactions are very sensitive to some structural features, such as a negatively charged carboxylate group attached to the ferrocene residue, which may completely disrupt the stability of the complexes.

Kano K., Nishiyabu R., and Doi R. (2005) Novel behavior of O-methylated beta-cyclodextrins in inclusion of meso-tetraarylporphyrins. *J Org Chem* **70**, 3667-3673.

Abstract: [structure: see text] The mechanism for formation of extremely stable 1:2 inclusion complexes of water-soluble meso-tetraarylporphyrins with heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin (TMe-beta-CD) in aqueous solutions has been studied by means of NMR spectroscopy and isothermal titration calorimetry. To simplify the system, 5,10,15-tris(3,5-dicarboxylatophenyl)-20-phenylporphyrin (1) was used as a guest porphyrin, because 1 forms only a 1:1 inclusion complex with cyclodextrin (CD). As host compounds, native beta-CD and the O-methylated-beta-CDs such as heptakis(2,3-di-O-methyl)- (2,3-DMe-beta-CD), heptakis(2,6-di-O-methyl)- (2,6-DMe-beta-CD), and TMe-beta-CDs were used. The thermodynamic parameters for complexation such as binding constants (K) and enthalpy (DeltaH degrees) and entropy changes (DeltaS degrees) were determined by means of isothermal titration calorimetry. The K value for complexation of 1 with CD increases in the order beta-CD ($K = (1.2 \pm 0.1) \times 10^3 \text{ M}^{-1}$) < 2,6-DMe-beta-CD ($(1.2 \pm 0.1) \times 10^4 \text{ M}^{-1}$) << TMe-beta-CD ($(6.9 \pm 0.4) \times 10^6 \text{ M}^{-1}$) < 2,3-DMe-beta-CD ($(8.5 \pm 0.5) \times 10^6 \text{ M}^{-1}$), indicating participation of the secondary OCH(3) groups in extremely strong complexation of 1 with CD. Complex formation of 1 with beta-CD and 2,6-DMe-beta-CD is an enthalpically and entropically favorable process, while that with TMe-beta-CD and 2,3-DMe-beta-CD is an enthalpically much more favorable but an entropically less favorable process. The thermodynamic parameters suggest that inclusion of 1 into the cavities of TMe-beta-CD and 2,3-DMe-beta-CD is promoted by van der Waals interactions, which are stronger than those in the cases of beta-CD and 2,6-DMe-beta-CD. ¹³C NMR spectra show that the conformations of both TMe-beta-CD and 2,3-DMe-beta-CD are altered upon inclusion of 1, while those of beta-CD and 2,6-DMe-beta-CD are mostly retained. On the basis of these results, it can be concluded that induced-fit type complexation of 1 with TMe-beta-CD and 2,3-DMe-beta-CD causes extremely strong binding of the host to the guest.

Kano K., Kitagishi H., Dagallier C., Kodera M., Matsuo T., Hayashi T., Hisaeda Y., and Hirota S. (2006) Iron porphyrin-cyclodextrin supramolecular complex as a functional model of myoglobin in aqueous solution. *Inorg Chem* **45**, 4448-4460.

Abstract: The 1:1 inclusion complex of 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron(II) (Fe(II)TPPS) and an O-methylated beta-cyclodextrin dimer having a pyridine linker (1) binds dioxygen reversibly in aqueous solution. The O(2) adduct was very stable ($t_{1/2} = 30.1 \text{ h}$) at pH 7.0 and 25 degrees C. ESI-MS and NMR spectroscopic measurements and molecular mechanics (MM) calculations indicated the inclusion of the sulfonatophenyl groups at the 5- and 15-positions of Fe(III)TPPS or Fe(II)TPPS into two cyclodextrin moieties of 1 to form a supramolecular 1:1 complex (hemoCD1 for the Fe(II)TPPS complex), whose iron center is completely covered by two cyclodextrin moieties. Equilibrium measurements and laser flash photolysis provided the affinities (K) and rate constants for O(2) and CO binding of hemoCD1 ($k_{\text{O(2)}}(\text{on})$, $k_{\text{O(2)}}(\text{off})$, $k_{\text{CO}}(\text{on})$, and $k_{\text{CO}}(\text{off})$). The CO affinity relative to the O(2) affinity of hemoCD1 was abnormally high. Although resonance Raman spectra suggested weak back-bonding of $d(\pi)(\text{Fe}) \rightarrow \pi(\text{CO})$ and hence a weak CO-Fe bond, the CO adduct of hemoCD1 was very stable. The hydrophobic CO molecule dissociated from CO-hemoCD1 hardly breaks free from a shallow cleft in hemoCD1 surrounded by an aqueous bulk phase leading to fast rebinding of CO to hemoCD1. Isothermal titration calorimetry furnished the association constant (K(O2)), DeltaH degrees, and DeltaS degrees for O(2) association to be $(2.71 \pm 0.51) \times 10^4 \text{ M}^{-1}$, $-65.2 \pm 4.4 \text{ kJ mol}^{-1}$, and $-133.9 \pm 16.1 \text{ J mol}^{-1}$ K⁻¹, respectively. The autoxidation of oxy-hemoCD1 was accelerated by H(+) and OH(-). The inorganic anions also accelerated the autoxidation of oxy-hemoCD1. The O(2)-Fe(II) bond is equivalent to the O(2)^(*)-Fe(III) bond, which is attacked by the inorganic anions or the water molecule to produce met-hemoCD1 and a superoxide anion.

Koushik K. N., Bandi N., and Kompella U. B. (2001) Interaction of [D-Trp6, Des-Gly10] LHRH ethylamide and hydroxy propyl beta-cyclodextrin (HPbetaCD): thermodynamics of interaction and protection from degradation by alpha-chymotrypsin. *Pharm Dev Technol* **6**, 595-606.

Abstract: PURPOSE: The purpose of this study is to investigate the mechanisms and thermodynamics of the interaction between hydroxypropyl beta-cyclodextrin (HPbetaCD) and [D-Trp6, des-Gly10] LHRH ethylamide (deslorelin), a peptide drug. METHODS: We used UV and fluorescence spectroscopy to study the interaction of HPbetaCD and deslorelin. Circular dichroism was used to study the conformational changes induced in deslorelin upon interaction with HP beta CD. The thermodynamics of the interaction of deslorelin and HPbetaCD was studied using isothermal titration calorimetry (ITC). We also determined the effect of HPbetaCD on the degradation of deslorelin by alpha-chymotrypsin. RESULTS: UV and fluorescence spectroscopy indicated that HPbetaD induced a change in polarity of the environment surrounding the chromophores of deslorelin. Wavelength selective fluorescence indicated an increase in the fluorescence polarization of deslorelin with an increase in excitation wavelength in the presence of HPbetaCD suggesting that tryptophan is present in a media of reduced mobility. Circular dichroism studies indicated that HPbetaCD stabilizes the conformation of deslorelin. In addition, ITC indicated an exothermic reaction between deslorelin and HPbetaCD with a low enthalpy of binding of approximately -600 cal/mol and a binding affinity of approximately $-1.25 \times 10^2 \text{ M}^{-1}$. Finally, the rate of degradation of deslorelin by alpha-chymotrypsin was decreased by 33% in the presence of HPbetaCD. CONCLUSIONS: These results indicate that there is an interaction between HPbetaCD and deslorelin, which involves the inclusion of aromatic amino acids of deslorelin into the hydrophobic cavity of the cyclodextrin. This inclusion, providing steric hindrance, may be one of the mechanisms by which HPbetaCD reduces enzymatic hydrolysis of deslorelin.

Kriz Z., Koca J., Imberty A., Charlot A., and Auzely-Velty R. (2003) Investigation of the complexation of (+)-catechin by beta-cyclodextrin by a combination of NMR, microcalorimetry and molecular modeling techniques. *Org Biomol Chem* **1**, 2590-2595.

Abstract: (+)-Catechin is a polyphenolic compound of natural origin that presents anti-oxidant properties of interest for therapeutics or cosmetics uses. Preliminary studies on inclusion into cyclodextrin cavities yielded contradictory results both for the quantitative (affinity constant) and qualitative description of the interaction. By a combination of several experimental and theoretical methods, the present study resolved the previous ambiguities about the interaction between (+)-catechin and beta-cyclodextrin. Thermodynamic data measured by isothermal titration calorimetry demonstrate that the binding is enthalpy driven. Excellent agreement has been obtained for the measurement of the association constant by NMR and microcalorimetry. The several docking modes obtained by systematic docking studies have been compared to intermolecular contacts measured by NMR and the overall geometry of the complex can be proposed.

Liu X. M., Lee H. T., Reinhardt R. A., Marky L. A. and Wang D. (2007) Novel biomineral-binding cyclodextrins for controlled drug delivery in the oral cavity. *J Control Release* **122**, 54-62.

Abstract: A biomineral-binding alendronate-beta-cyclodextrin conjugate (ALN-beta-CD, Fig. 1) was developed as a novel drug delivery system. "Click" chemistry was used in conjugation of alendronate (ALN) to beta-cyclodextrin (beta-CD). The delivery system shows very strong binding to hydroxyapatite (HA, main component of tooth enamel). Its ability in forming molecular inclusion complex with dexamethsone (Dex, model drug) was investigated independently with phase solubility experiments, isothermal titration calorimetry (ITC), Job plot and (1)H NMR. The stoichiometry of ALN-beta-CD/Dex molecular complex was determined as 1:1, and the binding constant of the complex obtained from ITC study is $3.8 \times 10^3 \text{ M}^{-1}$, which is similar to the binding constant of beta-CD/Dex. In vitro data indicate that the ALN-beta-CD/Dex complex bound to HA could gradually release Dex upon repeated extraction with phosphate buffer saline (PBS). This novel drug delivery system may have great potential in improving treatment of diseases in the oral cavity.

Liu Y., Yang Y. W., Yang E. C., and Guan X. D. (2004) Molecular recognition thermodynamics and structural elucidation of interactions between steroids and bridged bis(beta-cyclodextrin)s. *J Org Chem* **69**, 6590-6602.

Abstract: A series of bridged bis(beta-cyclodextrin(CD))s (2-7) were synthesized, i.e., bridged bis(beta-CD)s 2 and 3 bearing binaphthyl or biquinoline tethers and bridged bis(beta-CD)s 4-7 possessing dithiobis(benzoyl) tether, and their complex stability constants (KS), enthalpy (ΔH degrees), and entropy

changes (ΔS degrees) for the 1:2 inclusion complexation with representative steroids, deoxycholate, cholate, glycocholate, and taurocholate, have been determined in an aqueous phosphate buffer solution of pH 7.20 at 298.15 K by means of titration microcalorimetry. The original conformations of bridged bis(beta-cyclodextrin)s were investigated by circular dichroism and 1H ROESY spectroscopy. Structures of the inclusion complexes between steroids and bridged bis(beta-CD)s in solution were elucidated by 2D NMR experiments, indicating that anionic groups of two steroid molecules penetrate, respectively, into the two hydrophobic CD cavities in one 6,6'-bridged bis(beta-CD) molecule from the secondary rim to give a 1:2 binding mode upon inclusion complexation. The results obtained from titration microcalorimetry and 2D NMR experiments jointly demonstrate that bridged bis(beta-CD)s 2, 3 and 5-7 tethered by protonated amino group possessing different substituted groups can enhance not only the molecular binding ability toward steroids by electrostatic interaction but also molecular selectivity. Thermodynamically, the resulting 1:2 bis(beta-CD)-steroid complexes are formed by an enthalpy-driven process, accompanied by smaller entropy loss. The increased complex stability mainly results from enthalpy gain, accompanied by large conformational change and extensive desolvation effects for the 1:2 inclusion complexation between bis(beta-CD)s and steroids.

Liu Y., Zhao Y. L., Zhang H. Y., Yang E. C., and Guan X. D. (2004) Binding ability and assembly behavior of beta-cyclodextrin complexes with 2,2'-dipyridine and 4,4'-dipyridine. *J Org Chem* **69**, 3383-3390.

Abstract: Two channel-type supramolecular aggregations 1 and 2 were prepared by the inclusion complex of beta-cyclodextrin with 2,2'-dipyridine and 4,4'-dipyridine, respectively, and their binding ability and assembly behavior were investigated comprehensively by X-ray crystallography, (1H) NMR, circular dichroism spectra, and microcalorimetric titration in solution and the solid state. The obtained results revealed that the hydrogen bonds and pi-pi stacking interactions are crucial factors for the formation of the molecular aggregations containing beta-cyclodextrin and dipyridines. The disparity of nitrogen atom position in dipyridines leads not only to the distinct crystal system and space group, i.e., monoclinic system (C2) for 1 and triclinic system (P-1) for 2, but also different binding modes and thermodynamical parameters upon complexation of 2,2'-dipyridine and 4,4'-dipyridine with beta-cyclodextrin in aqueous solution.

Liu Y., Yang E. C., Yang Y. W., Zhang H. Y., Fan Z., Ding F., and Cao R. (2004) Thermodynamics of the molecular and chiral recognition of cycloalkanols and camphor by modified beta-cyclodextrins possessing simple aromatic tethers. *J Org Chem* **69**, 173-180.

Abstract: The complex stability constants ($K(S)$) and thermodynamic parameters (ΔG degrees, ΔH degrees, and ΔS degrees) for 1:1 inclusion complexation of beta-cyclodextrin (beta-CD) derivatives, 6-O-phenyl-beta-CD (2) 6-O-(4-formyl-phenyl)-beta-CD (3), 6-O-(4-nitrophenyl)-beta-CD (4), 6-O-(4-bromophenyl)-beta-CD (5), 6-O-(4-chlorophenyl)-beta-CD (6), and 6-O-(4-hydroxybenzoyl)-beta-CD (7) with representative guest molecules, cyclic alcohols (cyclopentanol, cyclohexanol, cycloheptanol, cyclooctanol), (+/-)-borneol, and (+/-)-camphor, have been determined by means of titration microcalorimetry in an aqueous phosphate buffer solution (pH = 7.20) at 298.15 K. The results obtained indicate that the introduction to beta-CD of an aromatic ring bearing different substituent groups significantly enhances the molecular binding ability and moderately alters the chiral discrimination ability for the guests examined here, displaying the highest enantioselectivity of up to 4.01 for the inclusion complexation of 6 with (+/-)-camphor. The enhanced molecular/chiral discrimination ability caused by derivatization is attributed solely to increased positive entropy changes due to the expanding hydrophobic interaction and desolvation effects. The binding modes of host-guest interactions derived from ROESY spectroscopy data show that the resulting complex of 4 and (+)-borneol possesses better induced-fit interaction as compared to (-)-borneol, which is responsible for the enhanced molecular/chiral recognition ability.

Liu Y., Li L., Li X. Y., Zhang H. Y., Wada T., and Inoue Y. (2003) Synthesis of phosphoryl-tethered beta-cyclodextrins and their molecular and chiral recognition thermodynamics. *J Org Chem* **68**, 3646-3657.

Abstract: Two novel phosphoryl-bridged bis- and tris(beta-cyclodextrin)s of different tether lengths, i.e., bis[m-(N-(6-cyclodextryl)-2-aminoethylaminosulfonyl)phenyl]-m-(chlorosulfonyl)phenylphosphine oxide (5) and tris[m-(N-(6-cyclodextryl)-8-amino-3,6-diazaoctylaminosulfonyl)phenyl]phosphine oxide (6), have been synthesized by reactions of 6-oligo(ethylenediamino)-6-deoxy-beta-cyclodextrins with tris[m-

(chlorosulfonyl)phenyl]phosphine oxide. The complex stability constants ($K(S)$), standard molar enthalpy (ΔH degrees), and entropy changes (ΔS degrees) were determined at 25 degrees C for the inclusion complexation of phosphoryl-modified bis- and tris-cyclodextrins (5 and 6, respectively), mono[6-O-(ethoxyhydroxyphosphoryl)]-beta-cyclodextrin (2), mono[6-O-(diethylamino-ethoxyphosphoryl)]-beta-cyclodextrin (3), and mono[6-O-(diphenoxyphosphoryl)]-beta-cyclodextrin (4) with representative alicyclic and N-Cbz-D/L-alanine guests in 0.1 M phosphate buffer solution at pH 7.2 by means of titration microcalorimetry. The thermodynamic parameters obtained indicate that the charge-dipole interaction between the phosphoryl moiety and the negatively charged guests, as well as the conformational difference of modified beta-cyclodextrins in aqueous solution, significantly contribute to the inclusion complexation and the enhanced chiral discrimination. The interactions and binding modes between the hosts and chiral guests were further studied by two-dimensional NMR spectroscopy to elucidate the influence of the structural features of hosts on their increased chiral recognition ability and to establish the correlation between the conformation of the resulting complexes and the thermodynamic parameters obtained.

Liu Y., Fan Z., Zhang H. Y., Yang Y. W., Ding F., Liu S. X., Wu X., Wada T., and Inoue Y. (2003) Supramolecular self-assemblies of beta-cyclodextrins with aromatic tethers: factors governing the helical columnar versus linear channel superstructures. *J Org Chem* **68**, 8345-8352.

Abstract: A series of 6-O-(p-substituted phenyl)-modified beta-cyclodextrin derivatives, i.e., 6-O-(4-bromophenyl)-beta-CD (1), 6-O-(4-nitrophenyl)-beta-CD (2), 6-O-(4-formylphenyl)-beta-CD (3), 6-phenylselenenyl-6-deoxy-beta-CD (4), and 6-O-(4-hydroxybenzoyl)-beta-CD (5), were synthesized, and their inclusion complexation behavior in aqueous solution and self-assembling behavior in the solid state were comparatively studied by NMR spectroscopy, microcalorimetry, crystallography, and scanning tunneling microscopy. Interestingly, (seleno)ethers 1-4 and ester 5 displayed distinctly different self-assembling behavior in the solid state, affording a successively threading head-to-tail polymeric helical structure for the (seleno)ethers or a mutually penetrating tail-to-tail dimeric columnar channel structure for the ester. Combining the present and previous structures reported for the relevant beta-CD derivatives, we further deduce that the pivot heteroatom, through which the aromatic substituent is tethered to beta-CD, plays a critical role in determining the helix structure, endowing the 2-fold and 4-fold axes to the N/O- and S/Se-pivoted beta-CD aggregates, respectively. This means that one can control the self-assembling orientation, alignment, and helicity in the solid state by finely tuning the pivot atom and the tether length. Further NMR and calorimetric studies on the self-assembling behavior in aqueous solution revealed that the dimerization step is the key to the formation of linear polymeric supramolecular architecture, which is driven by favorable entropic contributions.

Liu Y., Zhang Q. and Chen Y. (2007) Spectrophotometric and calorimetric titration studies on molecular recognition of camphor and borneol by nucleobase-modified beta-cyclodextrins. *J Phys. Chem B* **111**, 12211-12218.

Abstract: A series of modified beta-cyclodextrins with nucleobase substituents, that is, mono(6-ade-6-deoxy)-beta-cyclodextrin (2) and mono(6-ura-6-deoxy)-beta-cyclodextrin (3) as well as mono(6-thy-6-deoxy)-beta-cyclodextrin (4), were selected as molecular receptors to investigate their conformation and inclusion complexation behaviors with some chiral molecules, that is, (+)-camphor, (-)-camphor, (+)-borneol, and (-)-borneol, by spectrophotometric and microcalorimetric titrations in aqueous phosphate buffer solution (pH 7.2) at 298.15 K. Circular dichroism and NMR studies demonstrated that these nucleobase-modified beta-cyclodextrins adopted a co-inclusion mode upon complexation with guest molecules; that is, the originally self-included nucleobase substituents of the host did not move out from the beta-cyclodextrin cavity, but coexisted with guest molecule in the beta-cyclodextrin cavity upon inclusion complexation. Significantly, these nucleobase-modified beta-cyclodextrins efficiently enhanced the molecular binding ability and the chiral recognition ability of native beta-cyclodextrin, displaying enantioselectivity up to 3.7 for (+)-camphor/(-)-camphor pair by 2 and 3.5 for (-)-borneol/(+)-borneol pair by 3. The enhanced molecular/chiral recognition abilities of 2-4 toward (+/-)-camphor were mainly attributed to the increased entropic gains due to the extensive desolvation effects, while the favorable enthalpic gains originating from the good size-fit relationship as well as the hydrogen bond interactions between host and guest result in the enhanced molecular/chiral recognition abilities of 2-4 toward (+/-)-borneol.

Liu Y., Li X. Y., Zhang H. Y., Li C. J. and Ding F. (2007) Cyclodextrin-driven movement of cucurbit[7]uril. *J Org. Chem* **72**, 3640-3645.

Abstract: The movement of cucurbit[7]uril (CB[7]) driven by alpha-cyclodextrin (alpha-CD) is investigated by various experimental techniques including NMR, ESI-MS, UV-vis, and ITC. CB[7] can form stable pseudorotaxanes with N-methyl-N'-octyl-4,4'-bipyridinium (MVO²⁺) and N,N'-dioctyl-4,4'-bipyridinium (OV²⁺) dication in aqueous solution. CB[7] shuttles between the octyl and bipyridinium moieties in MVO²⁺, but docks at one of the octyl moieties in OV²⁺. The addition of alpha-CD pushes CB[7] from the octyl moiety of MVO²⁺ or OV²⁺ to the bipyridinium moiety. Thermodynamically, the movement of CB[7] is mainly driven by exothermic enthalpy changes coming from the complexation of the octyl moiety of MVO²⁺ or OV²⁺ with alpha-CD

Liu Y., Kang S., Chen Y., Cao R. and Shi J. (2007) Thermodynamics of molecular recognition of bile salts by 3,6'-(oligoethylenediamino-bridged) beta-cyclodextrin dimers. *Comb. Chem High Throughput. Screen.* **10**, 350-357.

Abstract: The molecular recognition behaviors of some representative bile salts by three 3,6'-bridged beta-cyclodextrin dimers with oligo(ethylenediamino) linkers in different lengths, i.e. 3,6'-(ethylenediamino-bridged) beta-cyclodextrin dimer (1), 3,6'-(diethylenetriamino-bridged) beta-cyclodextrin dimer (2), and 3,6'-(triethylenetetraamino-bridged) beta-cyclodextrin dimer (3), were investigated in aqueous phosphate buffer solution (pH 7.20) at 25 degrees C by means of 2D NMR spectroscopy and isothermal titration microcalorimetry. Owing to the cooperative host-linker-guest binding mode between host and guest, these 3,6'-bridged beta-cyclodextrin dimers showed significantly enhanced binding abilities and molecular selectivities as compared with native beta-cyclodextrin through the simultaneous contributions of hydrophobic, hydrogen bond, and electrostatic interactions. Thermodynamically, the inclusion complexations of these beta-cyclodextrin dimers with bile salts were mainly driven by large enthalpic gain, accompanied by slight to moderate entropic loss. An enthalpy-entropy compensation analysis demonstrated that these beta-cyclodextrin dimers experienced large conformational changes and extensive desolvation effect upon inclusion complexation with guest molecules.

Liu Y., Cao R., Chen Y. and He J. Y. (2008) Effect of beta-cyclodextrin charge type on the molecular recognition thermodynamics of reactions with (ferrocenylmethyl)dimethylaminium derivatives. *J Phys. Chem B* **112**, 1445-1450.

Abstract: Complex stability constants (KS), standard molar enthalpic changes (DeltaH degrees), and entropic changes (TDeltaS degrees) for the inclusion complexations of native beta-cyclodextrin (1) and two oppositely charged beta-cyclodextrins, i.e., mono(6-amino-6-deoxy)-beta-cyclodextrin (2) and mono[6-O-6-(4-carboxylphenyl)]-beta-cyclodextrin (3), with two (ferrocenylmethyl)dimethylaminium derivatives, i.e., FC⁴⁺Br⁽⁻⁾ and FC⁸⁺Br⁽⁻⁾, were determined at 25 degrees C in aqueous phosphate buffer solution (pH 7.20) by means of isothermal titration microcalorimetry (ITC). Cyclic voltammetry studies showed that the ferrocene groups of the guests were included in the beta-cyclodextrin cavity to form host-guest complexes. As compared with neutral beta-cyclodextrin, the positively charged host 2 showed decreased binding toward (ferrocenylmethyl)dimethylaminium guests. This was attributed to electrostatic repulsion, while the negatively charged host 3 displayed increased binding due to electrostatic attractions. Thermodynamically, the ionization of host CDs affects both enthalpic and entropic changes of host-guest complexations presumably by changing the hydrophobicity and the desolvation effect of hosts upon inclusion complexation. Moreover, the solvent effect was also discussed from the viewpoint of thermodynamics

Michels J. J., Huskens J., and Reinhoudt D. N. (2002) Noncovalent binding of sensitizers for lanthanide(III) luminescence in an EDTA-bis(beta-cyclodextrin) ligand. *J Am Chem Soc* **124**, 2056-2064.

Abstract: EDTA-linked beta-cyclodextrin dimer 3 was synthesized from EDTA bis(anhydride) 1 and mono(propylamino)-appended beta-cyclodextrin 2. p-tert-butylbenzoate 5, bound by the beta-cyclodextrin cavities of 3 with an association constant of 10⁴ M⁻¹ in water, acts as a sensitizer for the Eu(III) and Tb(III) complexes of 3. Luminescence spectroscopy, microcalorimetry, and Gd(III)-induced NMR relaxation rate measurements prove that 3 forms a 1:2 complex with 5 and that one of the beta-cyclodextrin-bound sensitizers coordinates to the EDTA-encapsulated Ln(III) ion. The Eu(III) complex of 3 forms strong 1:1 complexes (K approximately 10⁷ M⁻¹) with bis(propylamido adamantyl)-functionalized biphenyl sensitizers 7 and 8 in water. Both beta-cyclodextrins of 3 are involved in the binding of these guests. The amide

functionality adjacent to the biphenyl unit in 7 and 8 coordinates to the EDTA-encapsulated Ln(III) ion. For these biphenyl-based antennae both binding to beta-cyclodextrin and coordination to the Ln(III) center are crucial for efficient sensitization.

Mrozek J., Banecki B., Karolczak J., and Wiczak W. (2005) Influence of the separation of the charged groups and aromatic ring on interaction of tyrosine and phenylalanine analogues and derivatives with beta-cyclodextrin. *Biophys Chem* **116**, 237-250.

Abstract: Interactions of tyrosine and phenylalanine analogues with beta-cyclodextrin have been examined in terms of structural features of the ligand such as the separation of the charged amino group and aromatic ring, the presence of additional functional group attached to the amino or phenyl ring, and the presence of a charge on amino or carboxyl group, and steric effects using steady-state and time-resolved fluorescence spectroscopy and microcalorimetry. The studied aromatic amino acids possess low binding constant to beta-cyclodextrin, diversified with respect to the presence or absence of a substituent in para position of the phenyl ring. However, calculated, based on the global analysis of the fluorescence intensity decays, binding constants do not allow to estimate unequivocally the influence of the distance between the charged groups and phenol/phenyl ring on the inclusion complex stability because of their low diversification.

Mulder A., Jukovic A., van Leeuwen F. W., Kooijman H., Spek A. L., Huskens J., and Reinhoudt D. N. (2004) Photocontrolled release and uptake of a porphyrin guest by dithienylethene-tethered beta-cyclodextrin host dimers. *Chemistry* **10**, 1114-1123.

Abstract: Two photoswitchable dithienylethene-tethered beta-cyclodextrin dimers were synthesized to function as host molecules with an externally controllable binding affinity. The cyclodextrin cavities of these dimers are linked through their secondary sides by a photochromic dithienylethene unit that is connected to the secondary rim either directly (4) or through propyl spacers (9). Irradiation with light switches these dimers between a relatively flexible (open) and a rigid (closed) form. The binding properties of the dimers depend on the configuration of the dithienylethene spacer, as is shown by microcalorimetry performed with tetrakis-sulfonatophenyl porphyrin (TSPP) as a guest molecule. The differences in binding properties are most pronounced for the more rigid dimer 4, which binds TSPP 35 times more strongly in the open form (4 a) than in the closed form (4 b). The values found for the enthalpy of binding (ΔH°) indicate that this difference in binding is due to the loss of cooperativity between the two beta-cyclodextrin cavities in the closed form. Molecular modeling shows that 4 b is not able to bind TSPP effectively in both cyclodextrin cavities. The open and closed forms of the more flexible dimer 9 show no substantial difference in their binding of TSPP. Thermodynamic values indicative of strong binding of TSPP by two beta-cyclodextrin cavities were measured for both forms of the dimer, and molecular modeling confirms that both are flexible enough to tightly bind TSPP. The binding differences between the forms of dimer 4 allow the photocontrolled release and uptake of TSPP, which renders control of the ratio of complexed to free TSPP in solution possible.

Mulder A., Jukovic A., Huskens J., and Reinhoudt D. N. (2004) Bis(phenylthienyl)ethene-tethered beta-cyclodextrin dimers as photoswitchable hosts. *Org Biomol Chem* **2**, 1748-1755.

Abstract: Two beta-cyclodextrin dimers tethered by photoswitchable bis(phenylthienyl)ethene moieties were synthesized as potentially tunable receptor molecules. The cyclodextrin cavities of these dimers were linked via their secondary sides, with the photochromic bis(phenylthienyl)ethene unit either directly connected to the secondary rim (7) or via propyl spacers (10). By irradiation with light the dimers were reversibly switched between a relatively flexible (open) form and a rigid (closed) form. The photostationary states for both dimers consisted of 92% of the open and 8% of the closed form, enabling the nearly complete conversion between the two forms. The binding properties of the open and closed forms of dimers 7 and 10 were assessed by complexation studies with meso-tetrakis(4-sulfonatophenyl)porphyrin (TSPP) using isothermal titration calorimetry. For the rigidly tethered dimer 7, a factor 8 difference in binding affinity between the open and closed form of the dimer was found. This difference in binding affinity reflects the difference in enthalpy of binding for the two dimers, indicating that the beta-cyclodextrin cavities of the closed dimer 7b are spaced too far apart from each other by the rigid closed bis(phenylthienyl)ethene tether to cooperatively bind TSPP. The difference in binding affinity was sufficient to enable the phototriggered release of TSPP from dimer. The thermodynamic parameters obtained for dimer 10 suggested that the closed tether substantially contributes to the binding of TSPP. The open and closed form of dimer 10 bound TSPP with similar association constants, although the enthalpy of

binding for the complexation of TSPP by the closed form of dimer 10 was more favorable than that found for the open form of the dimer.

Muller A. and Wenz G. (2007) Thickness recognition of bolaamphiphiles by alpha-cyclodextrin. *Chemistry* **13**, 2218-2223.

Abstract: The minimum internal diameters of alpha-, beta-, and gamma-cyclodextrin were calculated by a space filling algorithm, MolShape, from the electron density maps created by semiempirical AM1 and PM3 calculations using Gaussian03. In addition, the minimum diameters of a series of dicationic bolaamphiphiles were calculated by MolShape as well. The calculated diameters of these hosts and guests allowed prognosis about the stabilities of the corresponding inclusion compounds. The experimental binding data, obtained by isothermal titration calorimetry (ITC), revealed indeed a very pronounced thickness recognition and correlate well with the calculated diameters.

Nilsson M., Valente A. J., Olofsson G., Soderman O. and Bonini M. (2008) Thermodynamic and kinetic characterization of host-guest association between bolaform surfactants and alpha- and beta-cyclodextrins. *J Phys. Chem B* **112**, 11310-11316.

Abstract: The thermodynamics and kinetics of formation of host-guest complexes between a series of bolaform surfactants of type $C_n Me_6(2+)2Br(-)$ ($n = 8, 10, \text{ and } 12$) and alpha-cyclodextrin and beta-cyclodextrin were studied with the aid of isothermal titration calorimetry (ITC) at 298.15 and 308.20 K. The association constant, the enthalpy, and the entropy of formation were determined. The obtained thermodynamic parameters are compared with parameters for the micelle formation of a related cationic surfactant. The difference in magnitude and sign between the parameters of the alpha-CD and beta-CD complexes is discussed based on the curvature of the cavity of the CD. We suggest that the water molecules inside the alpha-CD cavity are not able to maintain their hydrogen bond network. Upon complex formation these water molecules are expelled and reform their hydrogen bond network. The situation is different in the larger beta-CD cavity where water has the possibility of a more extensive hydrogen bonding. The kinetics for alpha-CD is slow, associated with high activation energies for both association and dissociation of the complex. The rates increased with a decrease in the number of methylene groups in the hydrocarbon chain. The slow kinetics is argued to originate from the fact that the charged headgroup needs to be pushed through a relative nonpolar cavity. A comparison is made with the Born energy

Parker K. M. and Stalcup A. M. (2008) Affinity capillary electrophoresis and isothermal titration calorimetry for the determination of fatty acid binding with beta-cyclodextrin. *J Chromatogr. A* **1204**, 171-182.

Abstract: Affinity capillary electrophoresis (ACE) and isothermal titration calorimetry (ITC) were used to investigate the binding interaction between several fatty acids (FAs) and beta-cyclodextrin (beta-CD). Within each method, steps taken to obtain accurate binding constants are discussed. The stoichiometry of interaction was revealed to be 1:1 regardless of FA chain length. The binding constants obtained using ACE were: octanoate, 6.4×10^2 ; 2-octenoate, 4.7×10^2 ; decanoate, 3.7×10^3 ; 9-decenoate, 1.8×10^3 and dodecanoate, 1.4×10^4 . The binding constants obtained from ITC were of the same order of magnitude, but were consistently greater than those from ACE. Thermodynamic data obtained using ITC are used to explain the observed trends in binding strength

Perry C. S., Charman S. A., Pranker R. J., Chiu F. C., Scanlon M. J., Chalmers D., and Charman W. N. (2006) The binding interaction of synthetic ozonide antimalarials with natural and modified beta-cyclodextrins. *J Pharm Sci* **95**, 146-158.

Abstract: The current studies were undertaken to explore the potential basis for a significant difference in the pharmacokinetic parameters after intravenous administration of a synthetic ozonide (OZ) antimalarial drug candidate (1) to rats when formulated in either Captisol (a sulfobutylether substituted beta-cyclodextrin derivative ((SBE)(7)-beta-CD)) or a buffered aqueous vehicle. It was suspected that the differences may have been due to failure of 1 to rapidly dissociate from the cyclodextrin complex in vivo, perhaps due to an unusually tight binding within the cyclodextrin cavity. To address this hypothesis, the binding of representative synthetic OZ antimalarial drug candidates (including 1) with beta-cyclodextrin and (SBE)(7)-beta-CD was investigated by isothermal titration calorimetry and phase solubility analysis. It was found that each of the OZ compounds exhibited an exceptionally high binding constant (approximately $10^6/M$) with both Cyclodextrins (CD). The nature of the complexation was investigated by

molecular dynamics simulations and NMR to explore the mechanisms, which generated such high binding constants. The data suggested that the most probable cause of the unusually high binding constants was a very close fit within the cyclodextrin cavity that resulted in more favourable changes in both the enthalpy and entropy of the binding interaction, compared to published data for other drugs.

Piatnitski E. L., Flowers R. A., and Deshayes K. (2000) Highly organized spherical hosts that bind organic guests in aqueous solution with μ molar affinity: microcalorimetry studies. *Chemistry* **6**, 999-1006.

Abstract: Two novel closed-shell hemicarcerand-like hosts with spherical cavities of 11 Å diameter that are soluble in aqueous solution were constructed. The binding of xylenes, aryl ethers, polyaromatic compounds, ferrocene derivatives, and bicyclic aliphatic compounds were examined by NMR spectroscopy and microcalorimetry. NMR binding studies indicated that binding depended upon guest hydrophobicity and shape. No binding was detected for guests in which a charge must be desolvated as part of inclusion or for guests that can not fit within the cavity of the host. Three complexes 2-naphthalene, 2-p-xylene, and 2-ferrocene were isolated and found to be indefinitely stable in the solid phase and in aqueous solution. The binding constants for these complexes are estimated to be greater than 10^8 M⁻¹. Thirteen guests were examined by microcalorimetry with binding constants ranging between 10^7 and 10^3 M⁻¹. A comparison of results obtained here with those from previous work with beta-cyclodextrin and cyclophane hosts, along with analysis of the entropy-enthalpy compensation data, indicate that there is a higher degree of guest desolvation with this host structure than with open-shell hosts. This accounts at least partially for the increase in affinity observed with these closed-shell hosts. Replacing a hydroxy group in the host portal with a hydrogen atom does not affect the binding constant, a finding consistent with the guest residing deeply buried within the host cavity. It was observed that aromatic guests are bound with higher affinity than aliphatic ones in agreement with results that point to the importance of London dispersion forces in the association of aromatic components in face-to-edge orientations. The correlation of changes in NMR chemical shift with microcalorimetry data supports a model in which increased CH- π interactions strengthen association between host and guest due to the dominant role of van der Waals dispersion forces. Remarkably, the binding constant for the 1,4 isomer of dimethoxybenzene is 32 times higher than for the 1,2 isomer, and even greater discrimination is observed between the xylene guests since the binding constant for p-xylene is 80 times greater than that for o-xylene. This discrimination between isomeric guests by a rigid host indicates that changes in specific hydrophobic interactions have substantial effects upon binding affinity.

Pineiro A., Banquy X., Perez-Casas S., Tovar E., Garcia A., Villa A., Amigo A., Mark A. E. and Costas M. (2007) On the characterization of host-guest complexes: surface tension, calorimetry, and molecular dynamics of cyclodextrins with a non-ionic surfactant. *J Phys. Chem B* **111**, 4383-4392.

Abstract: Three host-guest systems have been characterized using surface tension (σ), calorimetry, and molecular dynamics simulations (MD). The hosts were three native cyclodextrins (CD) and the guest the non-ionic carbohydrate surfactant octyl-beta-d-glucopyranoside. It is shown that, for any host-guest system, a rough screening of the most probable complex stoichiometries can be obtained in a model free form, using only calorimetric data. The σ data were analyzed using a model that includes a newly proposed adsorption isotherm. The equilibrium constants for several stoichiometries were simultaneously obtained through fitting the σ data. For alpha- and beta-CD, the predominant species is 1:1 and to a lesser extent 2:1, disregarding the existence of the 1:2. For gamma-CD, the 1:2 species dominates, the other two being also present. In an attempt to confirm these results, 10 ns MD simulations for each CD were performed using seven different starting conformations. The MD stable conformations agree with the results found from the experimental data. In one case, the spontaneous dissociation-formation of a complex was observed. Analysis of the trajectories indicates that hydrophobic interactions are primarily responsible for the formation and stability of the inclusion complexes. For the 2:1 species, intermolecular H-bonds between CD molecules result in a tight packed structure where their original truncated cone shape is lost in favor of a cylindrical geometry. Together, the results clearly demonstrate that the often used assumption of considering only a 1:1 species is inappropriate.

Rekharsky M. V. and Inoue Y. (2002) Solvent and guest isotope effects on complexation thermodynamics of alpha-, beta-, and 6-amino-6-deoxy-beta-cyclodextrins. *J Am Chem Soc* **124**, 12361-12371.

Abstract: The stability constant (K), standard free energy (ΔG degrees), enthalpy (ΔH degrees), and entropy changes ($T\Delta S$ degrees) for the complexation of native alpha- and beta-cyclodextrins (CDs) and 6-

amino-6-deoxy-beta-CD with more than 30 neutral, positively, and negatively charged guests, including seven fully or partially deuterated guests, have been determined in phosphate buffer solutions (pH/pD 6.9) of hydrogen oxide (H(2)O) or deuterium oxide (D(2)O) at 298.15 K by titration microcalorimetry. Upon complexation with these native and modified CDs, both nondeuterated and deuterated guests examined consistently exhibited higher affinities (by 5-20%) in D(2)O than in H(2)O. The quantitative affinity enhancement in D(2)O versus H(2)O directly correlates with the size and strength of the hydration shell around the charged/hydrophilic group of the guest. For that reason, negatively/positively charged guests, possessing a relatively large and strong hydration shell, afford smaller K(H2O)/K(D2O) ratios than those for neutral guests with a smaller and weaker hydration shell. Deuterated guests showed lower affinities (by 5-15%) than the relevant nondeuterated guests in both H(2)O and D(2)O, which is most likely ascribed to the lower ability of the C-D bond to produce induced dipoles and thus the reduced intracavity van der Waals interactions. The excellent enthalpy-entropy correlation obtained can be taken as evidence for the very limited conformational changes upon transfer of CD complexes from H(2)O to D(2)O.

Rekharsky M. V. and Inoue Y. (2002) Complexation and chiral recognition thermodynamics of 6-amino-6-deoxy-beta-cyclodextrin with anionic, cationic, and neutral chiral guests: counterbalance between van der Waals and coulombic interactions. *J Am Chem Soc* **124**, 813-826.

Abstract: The stability constant (K), standard free energy (ΔG degrees), enthalpy (ΔH degrees), and entropy changes (ΔS degrees) for the complexation of 6-amino-6-deoxy-beta-cyclodextrin with more than 50 negatively or positively charged as well as neutral guests, including 22 enantiomer pairs, have been determined in aqueous phosphate buffer (pH 6.9) at 298.15 K by titration microcalorimetry. The thermodynamic parameters obtained in this study and the relevant data for native beta-cyclodextrin indicate that the complexation and chiral discrimination behavior of the cationic host with charged guests are governed by the critical counterbalance between the electrostatic interactions of the charged groups in host and guest and the conventional intracavity interactions of the hydrophobic moiety of guest, such as hydrophobic, van der Waals, solvation/desolvation, and hydrogen-bonding interactions.

Rekharsky M. V., Yamamura H., Kawai M., and Inoue Y. (2003) Complexation and chiral recognition thermodynamics of gamma-cyclodextrin with N-acetyl- and N-carbobenzyloxy-dipeptides possessing two aromatic rings. *J Org Chem* **68**, 5228-5235.

Abstract: The stability constants (K) and the standard free energy (ΔG degrees), enthalpy (ΔH degrees), and entropy changes (ΔS degrees) for the complexation of gamma-cyclodextrin with 34 enantiomeric and diastereomeric N-acetyl- and N-carbobenzyloxy-d/l-dipeptides with two aromatic moieties were determined in aqueous buffer solution at 298.15 K by titration microcalorimetry. Chiral recognition of the enantiomeric dipeptide pairs by gamma-cyclodextrin was found to be fairly poor, exhibiting only small percentage differences in K, while the diastereomeric dipeptides were discriminated to much greater extent with affinity differences of up to 6-7 times. The complex structures of several selected pairs were elucidated by NMR techniques. Combining the microcalorimetric and NMR data, the complexation and chiral recognition behavior of gamma-cyclodextrin is discussed in particular in terms of the length, bulkiness, and flexibility of the tether connecting the two aromatic moieties in a guest.

Rekharsky M. V., Yamamura H., Kawai M., Osaka I., Arakawa R., Sato A., Ko Y. H., Selvapalam N., Kim K., and Inoue Y. (2006) Sequential formation of a ternary complex among dihexylammonium, cucurbit[6]uril, and cyclodextrin with positive cooperativity. *Org Lett* **8**, 815-818.

Abstract: [STRUCTURE: SEE TEXT] A unique ternary 1:1:1 cucurbit[6]uril (CB[6])-cyclodextrin (CD)-dihexylammonium (DHA) complex was designed and noncovalently synthesized in stepwise fashion: first, CB[6] interacts strongly with DHA to form a 1:1 complex; second, addition of CD into the solution of the 1:1 complex leads to the exclusive formation of the 1:1:1 ternary complex. The ternary complex was characterized by various experimental techniques including ITC, NMR, and ESI-MS.

Rodriguez-Perez A. I., Rodriguez-Tenreiro C., Alvarez-Lorenzo C., Taboada P., Concheiro A., and Torres-Labandeira J. J. (2006) Sertaconazole/hydroxypropyl-beta-cyclodextrin complexation: isothermal titration calorimetry and solubility approaches. *J Pharm Sci* **95**, 1751-1762.

Abstract: Complexation of sertaconazole (SN) with hydroxypropyl-beta-cyclodextrin (HP-beta-CD) was characterized by phase-solubility diagram measurements and isothermal calorimetry (ITC) in aqueous medium, and by differential scanning calorimetry (DSC), Raman spectroscopy and X-ray diffractometry in

solid state. The strongest interaction was observed at pH 1.2, at which two different 1:1 complexes can be formed depending on the hydrophobic ring of the drug involved in the process. At pH 5.8 and 7.4 the likelihood of 1:2 stoichiometry increases as a consequence of the simultaneous complexation of the nonprotonized imidazolyl and the dichlorophenyl groups. In the presence of 20% HP-beta-CD, SN solubility is enhanced by a factor of 116, 107, and 5 at pH 1.2, 5.8, and 7.4, respectively. Complexation enthalpy recorded by ITC showed the same tendency which confirms the practical interest of this technique for fast screening of the potential of CDs as drug solubilizers. Solubility and dissolution rate of the drug from compacts prepared with freeze-dried complexes were significantly greater than those obtained with SN powder or compacts made with physical blends.

Rogmann N., Seidel J., and Mischnick P. (2000) Formation of unexpected substitution patterns in sulfonylbutylation of cyclomaltoheptaose promoted by host-guest interaction. *Carbohydr Res* **327**, 269-274.

Abstract: The distribution of substituents in sulfonylbutylethers of cyclomaltoheptaose (beta-cyclodextrin) formed in aqueous medium has been determined by gas chromatography after hydrolysis and formation of the permethylated sulfonylfluoride derivatives. In contrast to other etherification reactions of beta-cyclodextrin, preferred substitution in position 3 of the glucose units has been detected. From ¹H NMR and microcalorimetric experiments, the formation of host-guest complexes by beta-cyclodextrin and the reagent 1,4-butane sultone in water became evident. This spatial preorganization presumably favors the reaction with the O-3. In contrast, in methyl sulfoxide preferred 2-O-alkylation was obtained, indicating that host-guest interaction does not influence regioselectivity in this solvent.

Ross P. D. and Rekharsky M. V. (1996) Thermodynamics of hydrogen bond and hydrophobic interactions in cyclodextrin complexes. *Biophys J* **71**, 2144-2154.

Abstract: Values of K , ΔG_o , ΔH_o , ΔS_o and ΔC_{po} for the binding reaction of small organic ligands forming 1:1 complexes with either alpha- or beta-cyclodextrin were obtained by titration calorimetry from 15 degrees C to 45 degrees C. A hydrogen bond or hydrophobic interaction was introduced by adding a single functional group to the ligand. The thermodynamics of binding with and without the added group are compared to estimate the contribution of the hydrogen bond or hydrophobic interaction. A change in the environment of a functional group is required to influence the binding thermodynamics, but molecular size-dependent solute-solvent interactions have no effect. For phenolic O-H-O hydrogen bond formation, ΔH_o varies from -2 to -1.4 kcal mol⁻¹ from 15 degrees C to 45 degrees C, and ΔC_p is increased by 18 cal K⁻¹ mol⁻¹. The hydrophobic interaction has an opposite effect: in alpha-cyclodextrin, $\Delta C_{po} = -13.3$ cal K⁻¹ mol⁻¹ per ligand -CH(2)-, identical to values found for the transfer of a -CH(2)-group from water to a nonpolar environment. At room temperature, the hydrogen bond and the -CH(2)-interaction each contribute about -600 cal mol⁻¹ to the stability (ΔG_o) of the complex. With increased temperature, the hydrogen bond stability decreases (i.e., hydrogen bonds "melt"), but the stability of the hydrophobic interaction remains essentially constant.

Schmidtchen F. P. (2002) The anatomy of the energetics of molecular recognition by calorimetry: chiral discrimination of camphor by alpha-cyclodextrin. *Chemistry* **8**, 3522-3529.

Abstract: The molecular recognition of both camphor enantiomers 2 with the chiral alpha-cyclodextrin (alpha-CD) 1 in water and D(2)O was examined by calorimetry. On the basis of statistically supported determinations the thermodynamics of 2:1 host-guest binding and chiral discrimination was evaluated. The energetic signature strongly supports hydrophobic interaction as the dominant driving force for camphor encapsulation by alpha-CD in water. The solvent isotope effect on the binding equilibrium served to dissect the experimental enthalpy ΔH_{ass} into direct interaction (ΔH_{intr}) and solvent reorganization (ΔH_{solv}) terms. From this analysis the mutual interaction of two cyclodextrin and one camphor molecules contributes only 25 % to the observed enthalpy of binding ΔH_{ass} , all the rest is attributed to solvent restructuring. Furthermore, the dramatic change in the pattern of thermodynamic state functions on solvent transfer from water to D(2)O is taken as compelling evidence for the involvement of water as a structural tectone in the supramolecular architecture of the 2:1 complex. As a corollary, bilateral host-guest interactions as conveyed by the lock-and-key metaphor of molecular recognition provide an inadequate description of this seemingly simple artificial host-guest system.

Soto T., V, Jover A., Garcia J. C., Galantini L., Meijide F., and Tato J. V. (2006) Thermodynamics of formation of host-guest supramolecular polymers. *J Am Chem Soc* **128**, 5728-5734.

Abstract: The interactions between three beta-cyclodextrin hosts (having 1-3 binding sites) and two adamantyl guests (having 1-2 binding sites) have been studied by ITC, ROESY, static and dynamic light scattering (SLS and DLS), and AFM and TEM techniques. The enthalpy and free energy values (determined from ITC experiments) evidence that the single interaction between one binding site of the guest and one binding site of the host is independent of the number of binding sites of the interacting species. The average values are ΔH degrees = -26.6 ± 2.3 kJ mol⁻¹ and ΔG degrees = -30.4 ± 3.2 kJ mol⁻¹, indicating that the process is mainly enthalpy driven. In all cases, the experimental molar ratio (from ITC experiments) agrees with the expected one from the number of binding sites of both the host and guest. The formation of polymer-like entities was demonstrated by SLS, DLS, AFM, and TEM measurements. The structure of polymers is linear when both the host and the guest are ditopic entities and dendritic (or Cayley tree type) when the host and the guest have three and two binding sites, respectively.

Sun D. Z., Li L., Qiu X. M., Liu F., and Yin B. L. (2006) Isothermal titration calorimetry and ¹H NMR studies on host-guest interaction of paeonol and two of its isomers with beta-cyclodextrin. *Int J Pharm* **316**, 7-13.

Abstract: Thermodynamic parameters of inclusion complex of beta-cyclodextrin (beta-CD) with paeonol and two of its isomers in aqueous solution have been determined with nano-watt-order isothermal titration calorimetry (ITC) and the host-guest inclusion structure has been investigated by using ¹H NMR spectra at 298.2 K. The analysis of thermodynamic data reveals that stoichiometry of beta-CD complex with paeonol (Pae) or acetovanillone (Ace) is 1:1 whereas the inclusion complex of beta-CD with 2-hydroxyl-5-methoxyacetophone (Hma) is in 1:1 coexistence with 2:1 stoichiometry. Further analysis indicates that formation of all the complexes is simultaneously driven by enthalpy and entropy, the inclusion complexation of Pae.beta-CD, Ace.beta-CD and Ham.beta-CD₂ is predominantly driven by entropy while Ham.beta-CD by enthalpy. The ¹H NMR spectra data provide clear evidence of the inclusion phenomena, which shows that the aromatic ring of the guest molecule insert itself into the torus from the narrow side of the cavity.

Terekhova I. V. (2008) Interactions of beta- and hydroxypropyl-beta-cyclodextrin with some purine alkaloids: thermodynamic study. *Curr. Drug Discov. Technol.* **5**, 168-172.

Abstract: The effect of native and hydroxypropylated beta-cyclodextrin on the solubility and activity of some purine alkaloids was examined. For this purpose, the solubility of purine alkaloids in pure water and in aqueous solutions of mentioned beta-cyclodextrins was determined at 298.15 K. Stability constants of inclusion complexes and their stoichiometry were obtained from solubility diagrams. Enthalpic characteristics of interactions occurring between beta-cyclodextrins and purine alkaloids in aqueous solution were calculated from the direct calorimetric measurements. It was found, that beta-cyclodextrin forms with purine alkaloids weak complexes which are stabilized only by the entropy term. Due to very low complexing affinity of both beta-cyclodextrins to studied purine alkaloids their solubilizing effect is insignificant. The influence of structure of purine alkaloids and beta-cyclodextrin on the thermodynamic parameters of interaction was discussed

Tong W. Q., Lach J. L., Chin T. F., and Guillory J. K. (1991) Structural effects on the binding of amine drugs with the diphenylmethyl functionality to cyclodextrins. I. A microcalorimetric study. *Pharm Res* **8**, 951-957.

Abstract: Solution calorimetry has been employed to evaluate the stability constants and enthalpy changes associated with complex formation between alpha-, beta, or gamma-cyclodextrin (CD) and a group of amine compounds having the diphenylmethyl functionality. Data from thermal titrations of the compounds were analyzed using nonlinear least squares. The standard free energy decrease accompanying the formation of inclusion complexes is generally due to a negative standard enthalpy change (ΔH degrees). The standard entropy change (ΔS degrees) was negative, except in the case of complexes formed with gamma-CD. Of the 13 compounds studied, only 2 formed complexes with 1:2 (compound:beta-CD) stoichiometry, terfenadine . HCl and cinnarizine . 2HCl. All the others formed 1:1 complexes. The structural effect on the stability constants, thermodynamics, and inclusion geometry was explored by relating the calorimetric results to the chemical structures of the guest molecules and the cavity sizes of the CD molecules. The results suggest that one of the phenyl groups of the diphenylmethyl functionality

resides in the CD cavity and is in van der Waals contact with the inside wall of the CD cavity. In the case of alpha- and beta-CDs, van der Waals interaction dominates in the stabilization. On the other hand, the interaction between these compounds and gamma-CD is largely entropically driven. Adiphenine . HCl forms a more stable complex with beta-CD than proadifen . HCl, suggesting that hydrogen bonding to the carbonyl oxygen by the hydroxyl group on the rim of the CD ring can influence the strength of the binding interaction.

Tong W. Q., Lach J. L., Chin T. F., and Guillory J. K. (1991) Structural effects on the binding of amine drugs with the diphenylmethyl functionality to cyclodextrins. II. A molecular modeling study. *Pharm Res* **8**, 1307-1312.

Abstract: Molecular modeling has been used to study the complexation between alpha, beta, or gamma-cyclodextrin (CD) and a group of amine compounds having the diphenylmethyl functionality. The computer program SYBYL 5.3 and the Tripos force field (version 5.2) were used for all the calculations. Three-dimensional structures of 13 amine compounds were built individually from their atoms, and CDs were built based on the X-ray crystallographic coordinates. The diphenylmethyl derivative-CD complexes were constructed and optimized. Based on the calculated binding energies accompanying the inclusion process, the preferred method of approach of the compounds to the cavities of the CD molecules, and the structural effects on the binding between amine compounds and three CDs were explored. The calculated binding energies exhibited a good correlation with the stability constants obtained from solution calorimetric titrations. The present study shows that for similar ligand molecules, the molecular modeling technique should enable us to visualize the structure of the inclusion complexes and will also assist us in determining the ability of a potential drug molecule to form a stable complex with CDs.

Tong W. Q., Lach J. L., Chin T. F., and Guillory J. K. (1991) Microcalorimetric investigation of the complexation between 2-hydroxypropyl-beta-cyclodextrin and amine drugs with the diphenylmethyl functionality. *J Pharm Biomed Anal* **9**, 1139-1146.

Abstract: Solution calorimetry has been employed to evaluate the stability constants and standard-enthalpy changes (ΔH degrees) associated with complex formation between 2-hydroxypropyl-beta-cyclodextrin (HP-beta-CD) and a group of amine compounds having the diphenylmethyl functionality in aqueous solution at 298.15 K. Data from microcalorimetric titrations of the compounds were analysed using a nonlinear least-squares method. Of the 12 compounds studied, only terfenadine.HCl formed a 1:2 (compound:HP-beta-CD) complex. All the others formed 1:1 complexes. The standard free energy decrease accompanying the formation of inclusion complexes is generally due to a negative ΔH degrees. This exothermic ΔH degrees can be interpreted as indicating that the binding forces for complexation include both the hydrophobic effect and strong van der Waals interactions. When a halogen substituent is in the aromatic ring, stability constants are higher and standard-entropy changes (ΔS degrees) become positive, suggesting greater hydrophobic interaction. Both adiphenine.HCl and proadifen.HCl form more stable complexes, suggesting that hydrogen bonding to the carbonyl oxygen by the hydroxyl-group on the rim of the CD ring could be an important contributor to the complexation. Substitution on the aliphatic carbon of the diphenylmethyl group was also found to be important in determining the ability of compounds to bind with HP-beta-CD. The independence of the thermodynamic constants on the degree of protonation in the case of bifunctional amines indicates that the amine functional groups do not penetrate into the HP-beta-CD cavity.

Wang H., Cao R., Ke C. F., Liu Y., Wada T., and Inoue Y. (2005) Diastereomeric molecular recognition and binding behavior of bile acids by L/D-tryptophan-modified beta-cyclodextrins. *J Org Chem* **70**, 8703-8711.

Abstract: [reaction: see text] Binding behavior of L- and D-tryptophan-modified beta-cyclodextrins (L/D-Trp-beta-CD) (1 and 2) with four bile acids, i.e., cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA), has been investigated by fluorescence, circular dichroism, and 2D-NMR spectroscopies and fluorescence lifetime measurement, as well as isothermal titration microcalorimetry. From the induced circular dichroism (ICD) and 2D NMR spectra, it is deduced that the D-Trp moiety of 2 attached to beta-CD is more deeply self-included in the cavity than that of the antipodal L-Trp moiety of 1, indicating appreciably enantioselective binding of the chiral sidearm by beta-CD. Interestingly, the original difference in conformation between 1 and 2 led to quite a large difference in affinity toward DCA, giving 3.3 times higher binding ability for 2 than for 1. Thermodynamically, the inclusion complexation of 1 and 2

with bile acids was entirely driven by favorable enthalpy change (ΔH degrees) with accompanying negative entropy change (ΔS degrees). The stronger binding of bile acids by L/D-Trp-beta-CD is attributable to higher enthalpic gains. The combined use of the calorimetric and NMR ROESY spectral examinations revealed the correlation between the thermodynamic parameters and the role of sidearm conformation in modified beta-cyclodextrins.

Wenz G., Strassnig C., Thiele C., Engelke A., Morgenstern B. and Hegetschweiler K. (2008) Recognition of ionic guests by ionic beta-cyclodextrin derivatives. *Chemistry* **14**, 7202-7211.

Abstract: Inclusion compounds of cationic, anionic, and neutral p-substituted derivatives of tert-butylbenzene complexed in beta-cyclodextrin and its ionic 6-mono and 6-hepta derivatives were systematically investigated by isothermal titration calorimetry (ITC). All inclusion compounds showed 1:1 stoichiometry with binding constants ranging from 10 to 3×10^6 M⁻¹. The binding free energies could be subdivided into apolar and electrostatic contributions. The electrostatic interactions could be quantitatively described by Coulomb's law by taking into account the degree of protonation of hosts and guests, the orientations of the guests within the hosts, and ion shielding as described by the Debye-Huckel-Onsager theory. The orientations of the guests within the cyclodextrin cavities were determined by ROESY NMR spectroscopy

Wilson D., Perlson L., and Breslow R. (2003) Helical templating of oligopeptides by cyclodextrin dimers. *Bioorg Med Chem* **11**, 2649-2653.

Abstract: beta-cyclodextrin-based receptors were synthesized and tested for their ability to induce a helical fold in peptides bearing hydrophobic amino acid residues in the i, i+11- or i, i+14-positions. Circular dichroism experiments revealed that a dimeric beta-cyclodextrin receptor synthesized from a [1,1'-biphenyl]-4,4'-dithiol core demonstrated an ability to fold a designed peptide bearing the artificial amino acid L-p-t-butylphenylalanine in the i, i+11-positions, while other dimeric and monomeric receptors failed to do so. Titration studies were performed using both circular dichroism and calorimetry, the analysis of which yielded an apparent $K(a)$ on the order of 10^4 - 10^5 M⁻¹. However, no evidence could be obtained for helical folding with a peptide carrying tryptophan residues in place of the p-t-butylphenylalanine units. Our studies suggest that receptors of this type may be useful in molecular recognition of hydrophobic, already alpha-helical peptides in aqueous solution.

Wimmer R., Achmann F. L., Larsen K. L., and Petersen S. B. (2002) NMR diffusion as a novel tool for measuring the association constant between cyclodextrin and guest molecules. *Carbohydr Res* **337**, 841-849.

Abstract: In this paper we introduce the use of diffusion measurements by nuclear magnetic resonance (NMR) spectroscopy for determining association constants of weak and very weak interactions between cyclodextrin and guest molecules, as long as both the free and complexed guest molecules are soluble to an extent that allows good sensitivity in the NMR experiment. The experimental setup and data analysis is discussed for three different guest molecules: L-phenylalanine, L-leucine and L-valine, representing different strengths of interaction. The underlying assumptions are discussed and the scope of the method (range of K_a values, requirements to the guest molecule) are discussed. The method's main advantage is its general applicability independent of chromogenic or electrochemical properties of the guest molecule. Whereas calorimetric methods that exhibit a similar generality, are applicable mainly to strong interactions, NMR diffusion measurements are applicable to weaker interactions down to the theoretical limit of 1 M⁻¹, the upper limit for K_a values to be determined by it is approximately 200. A further advantage of the method is the low amount of sample needed. The method is in principle applicable to any case of molecular recognition between a host and guest molecule leading to weak interactions.

Wintgens V., oud-Mahammed S., Gref R., Bouteiller L. and Amiel C. (2008) Aqueous polysaccharide associations mediated by beta-cyclodextrin polymers. *Biomacromolecules*. **9**, 1434-1442.

Abstract: Macromolecular assemblies were elaborated by mixing in water hydrophobically modified dextrans (MDC(n)) and beta-cyclodextrin polymers (pbetaCD) interacting by inclusion complexation between the hydrophobic moieties of MDCn and the beta-cyclodextrin cavities of pbetaCD. Dextrans have been modified by grafting alkyl groups (C(n)) of varying chain lengths (n = 8-16) and grafting ratio (3-6 mol%). Different pbetaCD polymers were synthesized by polycondensation of beta-cyclodextrin and

epichlorohydrin. The polymer-polymer interactions have been studied by fluorimetry, isothermal titration microcalorimetry, phase diagrams, and viscosimetry. The viscoelastic properties of the temporary networks (in the semidilute range) have been studied by rheology. The interaction mechanisms between the MDCn and p β CD can be understood taking into account the strength of the interaction between the alkyl group and the beta-cyclodextrin cavity (mainly controlled by the alkyl chain length), the density of junctions between the chains (depending on the alkyl grafting density and the p β CD molecular weight), and additional cooperative effect (arising for high alkyl grafting density)

Yamamura H., Rekharsky M. V., Ishihara Y., Kawai M., and Inoue Y. (2004) Factors controlling the complex architecture of native and modified cyclodextrins with dipeptide (Z-Glu-Tyr) studied by microcalorimetry and NMR spectroscopy: critical effects of peripheral bis-trimethylamination and cavity size. *J Am Chem Soc* **126**, 14224-14233.

Abstract: Complex stability constant (K), standard free energy (ΔG degrees), reaction enthalpy (ΔH degrees), and entropy change ($T\Delta S$ degrees) for 1:1 inclusion complexation of the diastereomeric dipeptides Z-d/l-Glu-l-Tyr (Z = benzyloxycarbonyl) and its component amino acids (Z-d/l-Glu and N-Ac-Tyr) with native alpha-, beta-, and gamma-cyclodextrins (CDs) and A,X-modified bis(6-trimethylammonio-6-deoxy)-beta-CDs (AX-TMA(2)-beta-CDs) were determined in buffer solution (pH 6.9) at T = 298.15 K by isothermal titration microcalorimetry. Concurrent NMR spectral examinations revealed that the penetration mode and the resulting complex architecture are dramatically altered by the peripheral modification and also by the CD's cavity size. Upon complexation of the ditopic Z-Glu-Tyr guest, native alpha- and beta-CDs preferentially bind the Z's phenyl group, whereas AX-TMA(2)-beta-CDs predominantly include the Tyr's phenol moiety. In contrast, native gamma-CD includes both of the aromatic moieties simultaneously in the same cavity. Furthermore, for isomeric AB-, AC, and AD-TMA(2)-beta-CDs, an inversion of enantioselectivity and a switching of the penetration mode were observed, critically depending on the position of TMA substituents.

Zhao Y. L., Dichtel W. R., Trabolsi A., Saha S., Aprahamian I. and Stoddart J. F. (2008) A redox-switchable alpha-cyclodextrin-based [2]rotaxane. *J Am. Chem Soc.* **130**, 11294-11296.

Abstract: A bistable [2]rotaxane comprising an alpha-cyclodextrin (alpha-CD) ring and a dumbbell component containing a redox-active tetrathiafulvalene (TTF) ring system within its rod section has been synthesized using the Cu(I)-catalyzed azide-alkyne cycloaddition, and the redox-driven movements of the alpha-CD ring between the TTF and newly formed triazole ring systems have been elucidated. Microcalorimetric titrations on model complexes suggested that the alpha-CD ring prefers to reside on the TTF rather than on the triazole ring system by at least an order of magnitude. The fact that this situation does pertain in the bistable [2]rotaxane has not only been established quantitatively by electrochemical experiments and backed up by spectroscopic and chiroptical measurements but also been confirmed semiquantitatively by the recording of numerous cyclic voltammograms which point, along with the use of redox-active chemical reagents, to a mechanism of switching that involves the oxidation of the neutral TTF ring system to either its radical cationic (TTF*+) or dicationic (TTF2+) counterparts, whereupon the alpha-CD ring, moves along the dumbbell to encircle the triazole ring system. Since redox control by both chemical and electrochemical means is reversible, the switching by the bistable [2]rotaxane can be reversed on reduction of the TTF*+ or TTF2+ back to being a neutral TTF