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ORIGINAL ARTICLE

In silico strategies to describe the formation of the inclusion complex between β-cyclodextrin and β-naphtyloxyacetic acid: a preliminary step towards prediction of log *K*

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Abstract Ligand/cyclodextrin is a peculiar type of ligand/receptor system. We report the results of a docking procedure using β-naphtyloxyacetic acid as ligand and various models of β-cyclodextrin as receptor. The results indicate that docking strategies can successfully be applied to any ligand/cyclodextrin system with the final aim of predicting log *K* values.

Keywords β-Cyclodextrin · β-Naphtyloxyacetic acid · Docking · In silico · Inclusion complexes

Abbreviations

ADME-Tox	Absorption, Distribution, Metabolism, Excretion and Toxicity
CD	Cyclodextrin
CSD	Cambridge Structural Database
GB-SA	Generalized Born-Surface Area
RMS	Root Mean Square difference between two sets of atomic coordinates
MIF	Molecular Interaction Field

Introduction

Modern drug design requires that Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME-Tox) be addressed early in drug discovery [1]. Molecular host-guest based systems are well-known tools to optimize drug release in the body. Cyclodextrins (CDs) have been widely used as hosts to form inclusion complexes, and can improve the solubility and bioavailability of poorly water-soluble compounds [2], making complexation with CDs a very useful strategy for drug delivery [3–6]. Reliable in silico prediction of the stability constants (log *K*) is mandatory to improve the design of inclusion complexes [7].

In principle, log *K* can be estimated by using docking tools [8]. This study is one part of a more general computational project focused on determining log *K* between CDs, derivatized and not, and molecules of pharmaceutical interest. Here, we report the results obtained in describing the inclusion complex between β-naphtyloxyacetic acid as ligand and β-CD as a receptor, obtained through a docking procedure especially designed for investigating CDs inclusion complexes.

Methodology

β-CD structures

β-CD (code BCDEXD03) and the complex of β-CD with naphthyloxyacetic acid (code ODEJOW) were found in the Cambridge Structural Database (CSD, version 5.27; data updates January 2006). These compounds were checked with Mercury [9], saved in the Tripos mol2 format (necessary for subsequent calculations) and read in MOE [10] to carefully check the coordinates, delete co-crystallized water molecules, and add hydrogen atoms when necessary. Finally, the geometry of hydrogens was minimized under MMFF94x and GB-SA conditions [11, 12] (RMS

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gradient < 0.001Å). These minimization conditions were adopted throughout the study.

Eight β-CDs models (Table 1) were used: the first (**1a**) was simply extracted from the complex with β-naphthyloxyacetic acid; the second (**1b**) was obtained from the minimization of **1a**; **1c** is the crystallographic structure of β-CD (BCDEXD03); **1d** was obtained from the minimization of **1c**; **1e** was obtained from **1d** by manual rotation of the primary oxygens which were oriented towards the exterior of the cavity and subsequent minimization; **1f** was built using MOE Builder to obtain a symmetric β-CD and minimized after orienting the primary oxygens to point towards the interior of the cavity; **1g** and **1h** were the same as **1f** except for the orientation of the primary oxygens: these were oriented outwards with regard to the cavity (**1h**) and were oriented in an intermediate manner (three pointing inwards and four pointing outwards) (**1g**).

Ligand structure

After extraction from the crystallographic structure (code ODEJOW), β-naphthyloxyacetic acid was minimised before its use in the docking runs.

Geometrical descriptors

For each β-CD conformation, three centroids (Fig. 1A) were defined as dummy atoms by the MOE Builder: the first (named P) was due to the six oxygen atoms of the primary face, the second (C) to the six glycosidic oxygen atoms, and the third (S) to the 12 oxygen atoms of the secondary face.

For the ligand (Fig. 1B), the centroid of the distal aromatic portion was also defined (L) together with the centroid of the carboxylic moiety (M).

Molecular Interaction Fields (MIFs)

MIFs [13–15] were calculated using the program GRID [16]. Two probes were chosen, water (OH2) and the hydrophobic probe (DRY). Default parameters were utilized, except for the number of planes of grid points per Angstrom (NPLA directive) which was set to 2. Since the volume of MIFs varies with the energy cutoff value, –5.0 kcal/mol was taken as cutoff for the hydrophilic field and –0.05 kcal/mol for the hydrophobic field. An in-house program was then used to obtain the number of points proportional to the two MIFs volumes [17].

Docking

A modified MOE-Dock module, kindly provided by the Chemical Computing Group, was used. Briefly, a Genetic Algorithm search procedure was used with MOE default parameter (1500 generations per run, 3 mutation frequencies, 7 birthrates) and GB-SA conditions [11]. The ligand flexibility was taken into account, and a random initial orientation was used [18]. Each run (one run for each β-CD model) generated 100 poses. All the complexes present in the eight final databases were finally minimised.

The 100 poses were ranked by their E (the total energy of the complex) and the β-CD of the complex with the lowest E was used as a template to superpose the remaining 99 poses. For each pose the ligand RMS was calculated with respect to all the others (4950 RMS were thus calculated for each database). Two poses showing RMS < 0.8Å and differing in E by less than 3 kcal/mol were considered equal and thus only the one with lower E was retained. This screening produced for each β-CD model a database of poses (ranging from 61 to 87) significantly different one from the other which was analysed with the aid of the distance between centroids, as described in ''Geometrical descriptors'' (P, C, S, L and M, Fig. 1). Finally, to complete the analysis, the eight databases were merged together and the complexes were sorted by their E value.

All calculations were performed on a Linux based dualprocessor Appro1124 server and on standard PCs operating with Microsoft Windows XP.

Table 1 β-CDs used as a receptor in this study

Name	Description
1a	β-CD extracted from the complex with naphthyloxyacetic acid (no minimization)
1b	β-CD extracted from the complex with naphthyloxyacetic acid (MMFF94x and GB-SA)
1c	β-CD X-ray structure (no minimization)
1d	β-CD X-ray structure (MMFF94x and GB-SA)
1e	β-CD X-ray structure (MMFF94x and GB-SA) and all primary OHs pointing outwards with regard to the cavity
1f	Symmetric β-CD with primary OHs pointing inwards
1g	Symmetric β-CD with three primary OHs pointing inwards and four pointing outwards
1h	Symmetric β-CD with primary OHs pointing outwards

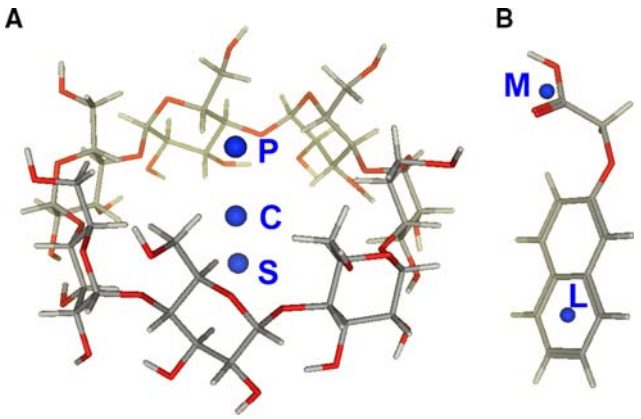

Fig. 1 3D chemical structures with relative centroids discussed in this study, (**A**) β-CD, (**B**) β-naphtyloxyacetic acid

Results and discussion

β-CD/β-naphthyloxyacetic acid as a model system for docking studies

Docking tools are generally applied to traditional ligand/receptor systems where the receptor is a protein with an active site located in its interior. In very simple terms, in the docking procedure the computer program places the ligand in the active site to predict the binding affinity of the ligand towards the receptor.

CDs exhibit low selectivity towards drugs, and CD inclusion complexes have low dissociation constants compared to receptor/drug complexes [19]. These characteristics, together with the similar size of receptor and ligand, make the CD/ligand system a particular type of ligand/receptor system, and mean that any standard computational procedure must be tailored to it. In this study, the β-CD/β-naphthyloxyacetic acid complex was selected to be submitted to computational runs, in order to determine a docking strategy tailored to it and able to reproduce experimental data, such as X-ray [20] and log K [21].

Once the procedure has been set up, at least in principle the docking method can be used to predict log K values for all CD/drug inclusion complexes.

Characterization of β-CD receptors

Since the receptor (i.e. the β-CD) is very flexible, (the active site is not constrained in any protein structure) the study had to take more than one conformation into account. Rather than performing a generic conformational analysis, from which to extract the most representative β-CD models, here we preferred to prepare eight β-CD models by modulating those features involved in the modifications induced on β-CD by complex formation [22]: (a) the shape of the cavity which tends to become oval in the presence of ligands and (b) the position of the primary oxygens, which in the X-ray structure of β-CD alone point at random towards the interior or the exterior of the cavity, whereas in crystallographic structures of β-CD complexes they always point outwards [17].

The eight final models are fully described in Methodology and listed in Table 1, whereas their geometrical characterization is summed up in Table 2. The cavity shape is described by d_{min} and d_{max} (respectively the shortest and the longest distance between the glycosidic oxygens and their centroid C (see Fig. 1A)) and

Table 2 Energy values, geometrical descriptors and MIFs numerical results for β-CD in its different conformations (see text)

	E [kcal/mol] (a)	d_{min} [Å] (b)	d_{max} [Å] (c)	CP [Å] (d)	CS [Å] (e)	DRY (f)	OH2 (g)	r (DRY/OH2) (h)
1a	652.08	4.94	5.17	2.88	2.12	415	1751	0.24
1b	585.70	4.82	5.02	1.77	2.51	476	1909	0.25
1c	620.89	4.81	5.17	3.13	2.10	55	1780	0.03
1d	584.26	4.80	4.98	2.80	2.36	122	1929	0.06
1e	584.00	4.80	4.98	2.33	2.36	415	1871	0.22
1f	572.56	5.00	5.05	3.66	2.41	138	1842	0.07
1g	572.63	5.00	5.05	3.14	2.41	163	1693	0.10
1h	579.11	5.00	5.05	2.60	2.41	451	1803	0.25

(a) β-CD energy calculated as described in the text

(b) The shortest distance between the glycosidic oxygens and their centroid C (see Fig. 1A)

(c) The longest distance between the glycosidic oxygens and their centroid C (see Fig. 1A)

(d) The distance between the centroids C and P (see Fig. 1A)

(e) The distance between the centroids C and S (see Fig. 1A)

(f) Number of final points of the MIF calculated with the DRY probe at –0.05kcal/mol

(g) Number of final points of the MIF calculated with the OH2 probe at –5kcal/mol

(h) Ratio between number of DRY points and number of OH2 points

thus **1f–1h** show a circular cavity, whereas the remaining models possess an oval cavity. The distance CS describes the position of the CD oxygens located on the secondary face, from which the entrance of the ligands should occur, whereas CP describes the position of the CD primary oxygens: the higher the CP the more the larger number of primary oxygens point inwards.

In previous studies, we have shown that the formation of an inclusion complex considerably changes some 3D molecular properties of CDs, as a consequence of changes in the orientation of primary oxygens [17, 23]. In particular, we found that the more lipophilic the ligand, the higher the lipophilic content around the primary face and the cavity. The ratio between hydrophilic and lipophilic content for models **1a–1h** is shown in Table 2, and suggests that, when the primary oxygens point outwards (**1a**, **1b**, **1e** and **1h**), the hydrophobic contribution is larger, as shown by the high r value and the increased presence of yellow regions in Fig. 2.

Finally, the lowest energies exhibited by the symmetric conformations (**1f–1h**) confirms that they are a reasonable model, regardless of their lack of experimental origin.

Docking results

The starting point in analysing the results is to define a criterion to judge whether poses found by the docking procedure are inside or outside the cavity. In this study, the ligand is considered to be inside the cavity when at least one of the distances MP, MC, MS, LP, LC, LS (Fig. 1A), as suggested by the X-ray data analysis, is below 2.5Å.

Docking results are reported in Table 3, where for each receptor model investigated (**1a–1h**), we report the total number of final poses (see Methodology) and those among them that lie outside or inside the β-CD cavity. The low percentage of poses inside the cavity for all models suggests that β-naphtyloxyacetic acid has little tendency to enter the β-CD cavity, which is in agreement with the low log K value (2.57 [21]). Preliminary results for larger compounds (e.g. imipramine and nicardipine) appear to confirm that ligands with higher log K enter the cavity to a greater extent.

The conformation of the receptor seems to have some influence on the entrance of the acid into the cavity since, in β-CD models with all primary oxygens pointing outwards (**1e** and **1h**), the number of ligands retrieved in the cavity is higher. This fact must be confirmed for ligands having greater affinity for the receptor.

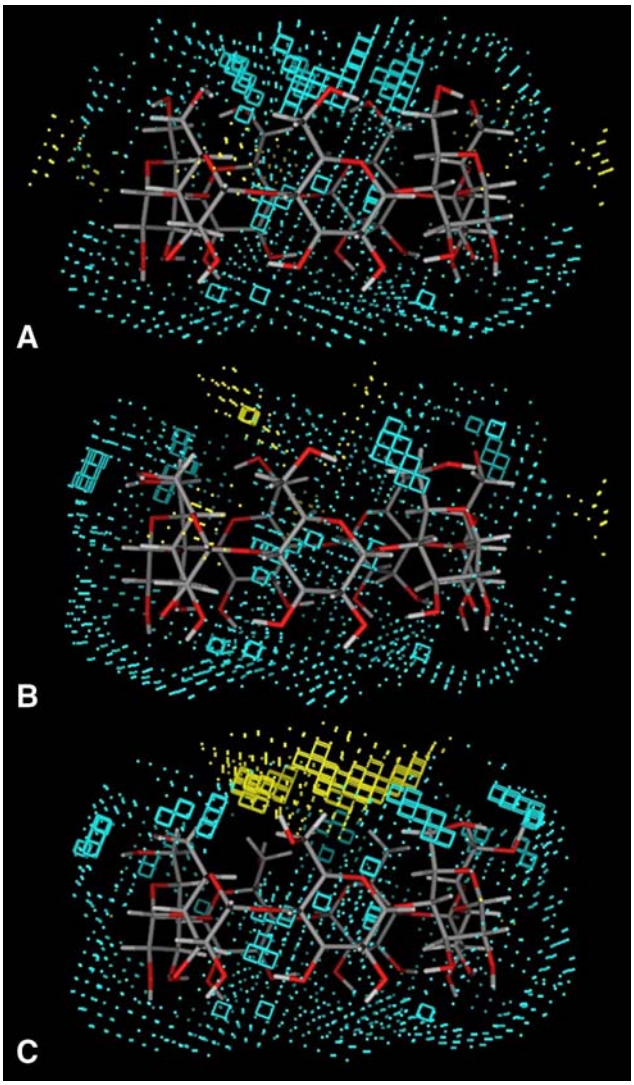

Fig. 2 GRID results visualization: hydrophilic regions (blue) at –5.0kcal/mol and hydrophobic regions (yellow) at –0.05kcal/mol. (**A**) **1f**, (**B**) **1g**, (**C**) **1h**

Table 3 Docking results

	Final poses (a)	Poses inside the cavity (%)	X-ray orientation (b)	Opposite X-ray orientation (c)
1a	87	1 (1)	1	0
1b	64	27 (42)	6	21
1c	61	4 (7)	2	2
1d	67	9 (13)	3	6
1e	65	11 (17)	2	9
1f	79	6 (8)	6	0
1g	80	10 (13)	9	1
1h	66	11 (17)	7	4

(a) Poses significantly different found by the docking procedure

(b) Poses located in the interior of the cavity and oriented in agreement with the X-ray data (code ODEJOW)

(c) Poses located in the interior of the cavity and oriented in the opposite way than the X-ray data (code ODEJOW)

From Table 3 it appears that, for all models (**1a**–**1h**), the molecules of β-naphtyloxyacetic acid which were found in the interior of the CD cavity are located in two main poses: one, in agreement with the X-ray data (Fig. 3A), has the naphthyl group is in the vicinity of the CD secondary oxygens, the other is in the opposite situation (Fig. 3C) with the polar moiety in the vicinity of CD primary oxygens. These results are in line with NMR data [20] indicating that, in solution, both these types of 1:1 complexes are found (similar behaviour is described in the literature for different CD/ligand systems [24–27]). Furthermore, this agreement with experimental data demonstrates that the proposed docking procedure is reliable, since it reproduces real situations.

An additional comment is that the different β-CD models influence the ratio between the two different poses (Table 3), and in particular that for symmetrical models (**1f**, **1g** and **1h**) show a preference for the X-ray-like pose. The reverse is true for results obtained using models **1c**, **1d**, and **1e**, which were obtained starting from the X-ray structure of the β-CD not involved in complex formation.

Finally, since the docking procedure requires final minimization of the complex, the results obtained from the eight models were merged into one database and sorted by increasing energy (E, the total energy of the complex). Analysis of this database reveals that the complex with the lowest energy (Fig. 3B) was obtained starting from **1g**, the ligand is in the same orientation as in the crystallographic structure (Fig. 3A) and the β-CD has lost its symmetry because of minimization (d_{min} = 4.31 Å, d_{max} = 5.32 Å). It must be pointed that the loss of symmetry also occurred for all complexes obtained from **1f**, **1g** and **1h**. In addition, in models derived from **1g**, the CD primary oxygens retain their orientation also after minimization.

The second-lowest-energy complex in the database differs from the first by less than 1 kcal/mol and has the ligand outside the CD cavity; thus also energy criteria confirm the low tendency of β-naphtyloxyacetic acid to form inclusion complexes with β-CD.

Of the following 79 complexes, 21 have the ligand with X-ray orientation, whereas 58 have the ligand outside the cavity. Complex 82 is the first to have the ligand in the opposite orientation to that of the X-ray (Fig. 3C); its E is 19 kcal/mol above that of the most stable complex (Fig. 3B).

Taken together, these results suggest that, in CD/ligand systems, the role of the energy values in the prediction of log K is not clear, partly because the contribution of entropy has been neglected. Conversely, the ratio between the number of ligand molecules that are found in the interior of the cavity and those that are found outside would appear to be the numerical docking result on which attention to

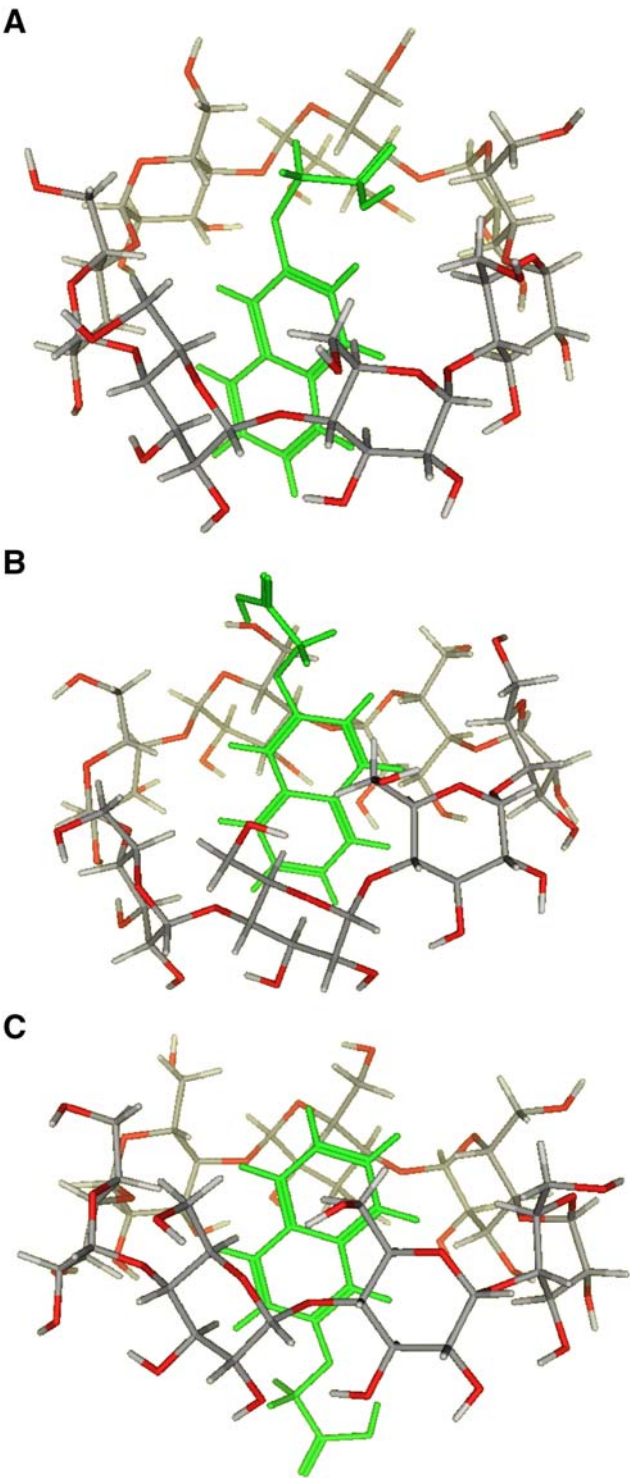

Fig. 3 Docking poses: the ligand is shown in green. (**A**) The crystallographic complex (code ODEJOW), (**B**) The complex of minimal energy E, (**C**) complex 82, that with lowest energy of those with the ligand in the opposite to the X-ray orientation

determine should be concentrated to determine a scoring function able to predict log K.

Conclusions

β-CD is a peculiar receptor, which can be used in docking studies provided that the computational procedure is tailored to it.

This study highlights that a symmetric β-CD built by computational tools with randomly oriented primary oxygens can be used as a representative receptor model of β-CD, since it is able to reproduce experimental NMR and crystallographic data for the systems β-CD/β-naphtyloxyacetic acid, chosen as reference complex. This result is of particular significance, since the computational building of derivatised CDs to use in further studies, will not have the support of crystallographic data.

Work is therefore in progress to extend this study, and should enable log K to be predicted for derivatised CDs with any ligand before their synthesis, topic of particular relevance in the design of new CD-based systems to be used in drug delivery.

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References

1. Van de Waterbeemd, H., Smith, D.A., Beaumont, K., Walker, D.K.: Property-based design: optimization of drug absorption and pharmacokinetics. J. Med. Chem. **44**, 1313–1333 (2001)
2. Davis, M.E., Brewster, M.E.: Cyclodextrin-based pharmaceutics: past, present and future. Nat. Rev. Drug Discov. **3**, 1023–1035 (2004)
3. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
4. Stella, V.J., Rajewski, R.A.: Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. **14**, 556–567 (1997)
5. Thompson, D.O.: Cyclodextrins-enabling excipients: their present and future use in pharmaceuticals. Crit. Rev. Ther. Drug Carr. Syst. **14**, 1–104 (1997)
6. Lichtenthaler, F.W., Immel, S.: On the hydrophobic characteristics of cyclodextrins: computer-aided visualization of molecular lipophilicity patterns. Liebigs Ann. 27–37 (1996)
7. Uccello-Barretta, G., Balzano, F., Paolino, D., Ciaccio, R., Guccione, S.: Combined NMR-crystallographic and modelling investigation of the inclusion of molsidomine into α-, β-, and γ-cyclodextrins. Bioorg. Med. Chem. **13**, 6502–6512 (2005)
8. Halperin, I., Ma, B., Wolfon, H., Nussinov, R.: Principles of docking: an overview of search algorithms and a guide to scoring functions. Protein Struct. Funct. Genet. **47**, 409–443 (2002)
9. Mercury, version 1.3., 2005, CCDC, Cambridge, UK, http://www.ccdc.cam.ac.uk/products/csd_system/mercury
10. MOE, version 2005.06,. 2005,. Chemical Computing Group, Montreal, Quebec Canada, http://www.chemcomp.com/
11. Qiu, D., Shenkin, P.S., Hollinger, F.P., Still, W.C.: The GB/SA continuum model for solvation. A fast analytical method for the calculation of approximate born radii. J. Phys. Chem. A **101**, 3005–3014 (1997)
12. Caron, G., Ermondi, G., Damiano, A., Novaroli, L., Tsinman, O., Ruell, J. A., Avdeef, A.: Ionization, lipophilicity, and molecular modeling to investigate permeability and other biological properties of amlodipine. Bioorg. Med. Chem. **12**, 6107–6118 (2004)
13. Goodford, P.J.: A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J. Med. Chem. **28**, 849–857 (1985)
14. Wade, R.C., Clark, K.J., Goodford, P.J.: Further development of hydrogen bond functions for use in determining energetically favorable binding sites on molecules of known structure. 1. Ligand probe groups with the ability to form two hydrogen bonds. J. Med. Chem. **36**, 140–147 (1993)
15. Wade, R.C., Goodford, P.J.: Further development of hydrogen bond functions for use in determining energetically favorable binding sites on molecules of known structure. 2. Ligand probe groups with the ability to form more than two hydrogen bonds. J. Med. Chem. **36**, 148–156 (1993)
16. GRID, version 22b, 2004, Molecular Discovery Ltd, West Way House, Elms Parade, Oxford, http://www.moldiscovery.com
17. Ermondi G., Anghilante C., Caron G.: A combined in silico strategy to describe the variation of some 3D molecular properties of β-cyclodextrin due to the formation of inclusion complexes. J. Mol. Graphics and Modelling **25**, 296–303 (2006)
18. Ermondi, G., Caron, G., Longo, D.: Design of a systematic docking tool and application to COX isozymes/NSAIDs complexes. J. Comput. Aid. Mol. Des. **18**, 683–696 (2004)
19. Houk, K.N., Leach, A.G., Kim, S., Zhang, X.: Binding affinities of host-guest, protein-ligand, and protein-transition-state complexes. Angew. Chem. Int. Ed. **42**, 4872–4897 (2003)
20. Kokkinou, A., Yannakopoulou, K., Mavridis, I.M., Mentzafos, D.: Structure of the complex of β-cyclodextrin with β-naphthyloxyacetic acid in the solid state and in aqueous solution. Carbohydrate Res. **332**, 85–94 (2001)
21. Rekharsky, M.V., Inoue, Y.: Complexation thermodynamics of cyclodextrins. Chem. Rev. **98**, 1875–1917 (1998)
22. Lipkowitz, K.B.: Applications of computational chemistry to the study of cyclodextrin. Chem. Rev. **98**, 1829–1873 (1998)
23. Caron G., Ermondi G.: Variations in MIFs content of α-cyclodextrins, caused by the formation of inclusion complexes, enables the complexes to be classified as Type 1 and Type 2. J. Mol. Graph Model, (2006). doi:10.1016/j.jmgm.2006.06.003
24. Uccello-Barretta, G., Balzano, F., Sicoli, G., Friglola, C., Aldana, I., Monge, A., Paolino, D., Guccione, S.:Combining NMR and molecular modelling in a drug delivery context: investigation of the multi-mode inclusion of a new NPY-5 antagonist bromobenzenesulfonamide into β-cyclodextrin. Bioorg. Med. Chem. **12**(2), 447–458 (2004)
25. Jara, F., Mascayano, C., Rezende, M.C., Tirapegui, C., Urzua, A.: A spectral and molecular dynamics simulation study of β-cyclodextrin inclusion complexes with

solvatochromic dyes derived from barbituric acid. J. Incl. Phenom. Macroc. Chem. **54**, 95–99 (2006)
26. Piel, G., Dive, G., Evrard, B., Van Hees, T., Henry de Hassonville, S., Delattre, L.: Molecular modeling study of b- and g-cyclodextrin complexes with miconazole. Eur. J. Pharm. Sci. **13**, 271–279 (2001)
27. Fernandes, C.M., Carvalho, R.A., Pereira da Costa, S., Veiga, F.J.B.: Multimodal molecular encapsulation of nicardipine hydrochloride by ***β***-cyclodextrin, hydroxypropyl-*β*-cyclodextrin and triacetyl-***β***- cyclodextrin in solution. Structural studies by ^{1}H NMR and ROESY experiments. Eur. J. Pharm. Sci. **18**, 285–296 (2003)