Published in final edited form as:

Food Biophys. 2013 September 1; 8(3): 216–222. doi:10.1007/s11483-013-9290-7.

The Interaction of Sorbitol with Caffeine in Aqueous Solution

Letizia Tavagnacco^{†,§,*}, John W. Brady[†], and Attilio Cesàro[§]

[†]Department of Food Science, Cornell University, Ithaca, NY 14853

[§]Department of Life Sciences, University of Trieste, Trieste, ITALY

Abstract

Molecular dynamics simulations were carried out on a system of caffeine interacting with the sugar alcohol sorbitol. The system examined had a caffeine concentration 0.083 m and a sugar concentration 1.08 m. The trajectories of all molecules in the system were collected over a period of 80 ns and analyzed to determine whether there is any tendency for sorbitol to bind to caffeine, and if so, by what mechanism. The results show that the sorbitol molecules have an affinity for the caffeine molecules and that the binding occurred by the interaction of the aliphatic hydrophobic protons of the sugar with the caffeine face. This intermolecular association *via* face-to-face stacking, as suggested by simulation studies, is similar to that found for sucrose and for D-glucose, which overwhelmingly exists in the pyranose ring chair form in aqueous solution, as well as for caffeine-caffeine association. The sorbitol molecules, however, exist as relatively extended chains and are, therefore, topologically quite different from the sugars sucrose and glucose. The comparison of the average conformation of sorbitol molecules bound to caffeine with that of molecules in the free state shows a substantial similarity.

Keywords

molecular dynamics simulations; caffeine; sorbitol; aqueous solution; caffeine-sugar binding; sorbitol conformation

Introduction

Caffeine is one of the most widely consumed psychostimulant substances in the world. It is mostly consumed in form of coffee and tea, which are the most popular drinks besides plain water. Currently available evidence supports the idea that caffeine enhances exercise performance primarily by acting on the central nervous system.¹ The presence and role of other components in caffeine-containing beverages and the highly complex mechanisms of caffeine action are still not fully understood and represent the principal objectives of many studies. For instance, although it is often reported that caffeine might negatively impact the cardiovascular system and metabolic control, this does not seem to be the case when caffeine is consumed in the form of coffee. It would be therefore a priority to study the effects of caffeine in the presence of the many other constituents of coffee or tea.

The physico-chemical characteristics of caffeine in mixture with natural and synthetic sugars or sweeteners (either of low or high molecular weight) are inadequately studied. Caffeine is only sparingly soluble in water at room temperature,^{2,3} and exhibits a significant tendency to self-associate in order to reduce its unfavorable interactions with the solvent.² This aggregation is a form of hydrophobic association, although it is atypical in that

* Author to whom correspondence should be addressed: lt287@cornell.edu, letizia.tavagnacco@phd.units.it, +39 040 558 3684;.

calorimetrically it is enthalpically-dominated,^{2,4} unlike the hydrophobic association of smaller species such as methane, which is entropy-dominated.⁵ As a result of this low solubility, caffeine also tends to associate with other species in solution, including chlorogenic acid^{6,7} and the sugars sucrose³ and glucose.

Recently we reported molecular dynamics (MD) simulation and nuclear magnetic resonance (NMR) studies of the association of these sugars with caffeine,⁸ as well as MD simulations of caffeine self-aggregation,⁹ and were able to characterize the role of water structuring in driving these associations. Interestingly, caffeine association with itself or with other species is apparently driven by the way in which its extended flat surface structures adjacent water molecules differently from that around smaller hydrophobic species like methane.¹⁰ When smaller non-polar solutes are in aqueous solution, adjacent water molecules orient so as to “straddle” the solute, pointing one of their O-H bonds directly away from the solute and directing neither of their protons or lone pairs directly at the solute, since that would involve the loss of a hydrogen bond.¹¹ By straddling the solute, the water molecules are able to “look past” the non-hydrogen bonding species and make hydrogen bonds to other water molecules. This structuring, with its attendant loss of rotational freedom, reduces the entropy, and the additional solvent structure results in an increase of the heat capacity. Association of two such non-polar species would liberate some of these structured water molecules, increasing their rotational freedom and entropy, so that in a calorimetry experiment the association would be characterized as entropy-dominated. This type of association also drives the insolubility of fats, since their fatty acid chains have small cross-sectional spatial dimensions and high curvatures, much like the methane molecule, so that water molecules can also straddle the individual methylene $-\text{CH}_2-$ groups in the fatty acid chains. Such structuring also contributes to the hydrophobic collapse of polypeptide chains in protein folding.⁵

However, recent theoretical studies suggest that more extended hydrophobic species would hydrate differently from smaller nonpolar molecules.^{5,10,12–15} As the hydrophobic surface becomes wider, it becomes impossible for a water molecule to straddle it in order to maintain its hydrogen bonding to other solvent molecules, as in the methane case, so first layer water molecules orient so as to direct one of their O-H bonds directly at the surface, since this arrangement sacrifices only one hydrogen bond instead of two or three. The flat surface of caffeine is just large enough to give rise to this effect, and when two caffeine molecules associate in solution, the release of this structured water allows the recovery of their full bulk hydrogen bonding capacity, and their binding is thus enthalpy-dominated.^{2,4}

D-Glucose, which overwhelmingly exists in the pyranose ring chair form in aqueous solution,¹⁶ will also bind to caffeine, as does sucrose as well.⁸ Simulation studies suggest that this association is also via face-to-face stacking. In the $^4\text{C}_1$ conformer of the α -D-glucopyranose ring form of D-glucose, all of the non-polar protons are in axial positions pointing either up or down,¹⁷ giving the top and bottom of the ring a hydrophobic character, particularly for the triad of H1–H3–H5 atoms. These atoms are held in their approximate relative positions by the rigidity of the ring structure, and thus play an important role in the interactions of glucose with other species. In simulation studies, there is a strong tendency for the H1–H3–H5 triad to stack against the caffeine face in solution. However, there is little or no tendency for glucose molecules to self-associate in a pure glucose aqueous solution.¹⁸ Neither glucose nor sorbitol exhibit any significant aggregation in aqueous solution,¹⁹ as both are very soluble and would be classified as osmolytes in an Hofmeister sense.²⁰

Sorbitol is the sugar alcohol that results from the reduction of the aldehyde group of D-glucose (Figure 1). Without this reactive aldehyde group, sugar alcohols do not form the stable five- and six-member rings that the aldoses can, and thus are topologically quite

different, existing as relatively extended chains. In sorbitol, the linear structure of the chain allows rotations around each C–C bond, potentially disrupting the development of any extended hydrophobic surface on the sugar alcohol. It is thus possible that such structural differences could affect the interactions of sugar alcohols with hydrophobic solutes, compared with the ring structures of the aldose sugars. However, torsional angles along the sorbitol chain are partially restricted, with some preference for linear extension, at least in the central part of the molecule. Although torsional bending at C2-C3 is commonly found in crystalline polymorphs of sorbitol²¹ previous investigation of the conformational features of sorbitol in water (at 3 m) suggested that a number of different conformers were significantly present at this relatively high concentration.²² The extended conformers were stabilized by a quite persistent intramolecular hydrogen bond between the hydroxyl groups of carbons C-2 and C-4. In another paper, Hatcher et al.²³ found similar results with some minor differences in the presence of other less populated bent conformations in the middle part of the chain. Given this potential conformation variability, the present simulations were conducted to determine whether there is any tendency for sorbitol to bind to caffeine, and if so, by what mechanism.

Methods

Molecular dynamics simulations were performed on a system consisting of a single caffeine molecule in a concentrated aqueous solution of sorbitol using the general molecular mechanics program CHARMM.^{24,25} The sorbitol molecules were modeled using the newly-developed polyol parameters in the CHARMM36 carbohydrate force field,²³ whereas the caffeine solute was modeled using the same force field employed previously.⁹ The water molecules were modeled using the TIP4P model.²⁶ Therefore, with respect to previous simulations on sorbitol reported in the literature,^{22,23} the present study introduces both a new force field and a new water model. The simulation was implemented following the general procedures previously used to simulate pure aqueous solutions of caffeine⁹ and of caffeine in glucose and sucrose solution.⁸ The periodic cubic box, generated by superimposition of the solute molecules onto an equilibrated water box, contained 1 caffeine molecule, 13 sorbitol molecules and 666 TIP4P water molecules. This system had a caffeine concentration of 0.083 m and a sorbitol concentration of 1.08 m. The size of the cubic box was rescaled to 28.40 Å to achieve an atom number density of 0.1030 atoms Å⁻³. The initial configuration was minimized with 500 steps of steepest descent minimization. The system was then heated from 0 K to 300 K over 6 ps and equilibrated for 1 ns with velocities being reassigned every 30 fs. Covalent bonds involving hydrogen atoms were kept fixed in length using the general SHAKE algorithm.²⁷ Van der Waals interactions were smoothly truncated on an atom-by-atom basis using switching functions from 10.5 to 11.5 Å. Electrostatic interactions were calculated using the particle-mesh Ewald method.²⁸ The system was studied in the NVE ensemble. Previous experience with similar binding systems has found that the choice of ensemble (NPT vs NVE) makes no difference in observed association tendencies.²⁹ The trajectory data were collected for 80 ns.

Results and Discussion

Caffeine-sorbitol binding

The statistics of the interaction of sorbitol molecules with the caffeine molecule have been calculated by evaluating the probability of the “binding events”. This provides a picture of the anisotropic structuring of the sorbitol relative to the caffeine, calculated by averaging the sugar atom positions over the whole trajectory of 80 ns. In practice, for each saved frame in the trajectory data, the instantaneous position of the caffeine molecule was mapped onto its initial position through a coordinate transformation; the same operation was then applied to all the sugar and water molecules. The transformed space was partitioned into cubes of 0.3

Å in length and the density of specific atoms of the sorbitol molecule in each cubic box was averaged over the whole trajectory.

Figure 2 displays contours of the sorbitol density relative to the caffeine molecule as obtained by taking different groups of atoms inside the sugar molecule. The red contour encloses regions of space in a caffeine-centered reference frame that have a carbon atom density that exceeds mean bulk density by a factor of 3.5 (the same factor is also applied to all other atomic species, see below). As can clearly be seen, there is a pronounced tendency for the sorbitol to associate with the flat surfaces of the caffeine. The absence of molecules in the plane of the ring seems related to no clear hydrogen-bonding affinity between sorbitol hydroxyls toward caffeine H-bond acceptors. The overall binding exhibits little significant qualitative difference from that observed for glucose in the sense that the sorbitol molecules interact with the flat faces and not with the peripheral hydrogen bond acceptor groups in the caffeine plane; there was negligible tendency for the sorbitol to out-compete with water molecules and hydrogen bond to the caffeine carbonyl oxygen and N9 atoms (Table 1). On the right side of Figure 2 the overlapping of the atom density maps calculated for different atom groups of the sorbitol molecules is displayed around the caffeine molecule. The high localization of the sorbitol atom density clouds and their “sandwich-like” shape indicates the existence of a preferred orientation in the interaction between the sorbitol molecule and the caffeine surface.

The additional detail shown in Figure 3, displaying the contour density map calculated for sorbitol hydroxyl proton atoms as seen from “above” the caffeine ring, indicates the presence of a specific orientation. Here, the clouds refer to the sorbitol hydroxyl protons that apparently align over the N9, N1 and N3 regions at a distance from the caffeine plane higher than the distance of the aliphatic protons. Some of the preferences for these sites may be due to the formation of hydrogen bonds with the caffeine acceptor groups. Table 1 shows the averaged number of hydrogen bonds that each caffeine acceptor group can make either with water or sorbitol donor groups averaged over the whole trajectory. The criteria used to define an hydrogen bond were a cutoff distance between heavy atoms of 3.4 Å and a cutoff angle of 150°. The caffeine hydrogen bonding atoms O2, O6, and N9 make approximately one hydrogen bond each, preferentially with water.

Figure 4 displays a representative snapshot of sorbitol molecules binding to the face of the caffeine solute. The sorbitol molecules are generally interacting with the purine face with their non-polar hydrogen atoms in contact, and with their hydrogen bonding hydroxyl groups pointing off to the sides in order to make hydrogen bonds to the solvent. As already noted, there was little observed tendency for the sorbitol molecules to hydrogen bond to the caffeine; Table 1 lists the averaged number of hydrogen bonds made by the polar groups of the caffeine to water and to the sorbitol. These atoms are hydrogen bonded to water molecules over 90% of the time, as is also true for the sorbitol.

Using the Gibbs equation, is possible to estimate the binding energy of the sorbitol molecules to the face of the caffeine as a simple equilibrium between the bound and unbound states, with the latter being characterized as the mean bulk sugar density far from the solute.

$$\Delta G = -RT \ln K \quad (1)$$

Since this energy is somewhat arbitrarily dependent on the boundary chosen to define the binding site, the estimated binding energy as a function of this cutoff choice is shown in Table 2. As can be seen, this energy is roughly 5.8 kJ/mol (1.2 kcal/mol), or 3 kJ/mol per

site, similar to the estimated binding energy for glucose, and does not depend strongly on the arbitrary choice of boundary for the binding site

Sorbitol orientation and conformation

As far as the orientation of the sorbitol chain in the complex is concerned, the analysis could be facilitated by the almost extended conformation of the sugar. Thus, the evolution of the end-to-end distances between C1 and C_n (with $n = 4, 5$ and 6) and their probability distribution have been evaluated. These results are plotted in Figure 5. The variation of the end to end distance between the carbon atoms of the sorbitol molecule with the time shown in Figure 5a represents the entire computed conformational history of the sugar molecule. While the trend of the C1-C6 distance is more variable, the C1-C4 and C1-C5 distances are quite stable with time and furthermore they undergo similar changes. Figure 5b illustrates details of the conformational behaviour of the sorbitol molecules through a comparison between sugar molecules interacting with ("bound to") the caffeine molecule and those "free" in the bulk of solution. Consistent with previous work,⁸ a sorbitol molecule interacts with the caffeine molecule when the distance between the two centres of mass is lower than 5 Å and the centre of mass of the sorbitol molecule fits in the plane described by the caffeine molecule. A sorbitol molecule is instead defined to be in the bulk solution when the distance between the centre of mass of the sorbitol molecule and the caffeine molecule is higher than 14 Å, in order to ensure the absence of direct interaction between the sorbitol molecule and the caffeine molecule.

The probability curves for the C1-C4 and C1-C5 distances for both bound and free sorbitol have bimodal distributions with maximum peaks centered around 3.9 Å and 5 Å, respectively. This indicates that the sorbitol molecules adopt an almost planar-zigzag conformation for about 90% of the time, while for the remaining part of the time the sugar molecule assumes a bent conformation as a consequence of the rotation around the C2-C3 bond. The probability distribution of the end to end distance calculated for the carbon atoms C1-C6 has a more complex shape that results from the convolution of two different distance contributions: the two population states of the C1-C5 chain, fully extended and tilted, and the three possible rotational states of the C4-C5 dihedral. The comparison between the probability distribution calculated for the sorbitol molecules interacting with the caffeine molecule and those in the bulk solution reveals that the association of the two solutes does not affect the conformation of the sorbitol molecule.

The high probability of an extended C1-C5 conformation supports the use of the vector C1-C5 as a geometrical parameter for assessing the orientation of sorbitol on the caffeine face. The results are reported in Figure 6, which displays the probability distributions of the cosine of the angle between the dipole moment vector of the caffeine molecule (see Figure 1) and the C1-C4 and C1-C5 vectors of the sorbitol molecule. The two almost equal maximum peaks that both the curves exhibit at $\cos\theta = -1$ and $\cos\theta = 1$ reveal that the sugar molecule tends to align its chain with the caffeine dipole moment (that is the major axis of caffeine). This orientation maximizes the aliphatic proton surface exposed to the caffeine hydrophobic surface, without preference for a parallel or antiparallel alignment. It is also evident from the figure that an alignment of the sorbitol molecule perpendicular to the caffeine major axis is also possible, although less favorable. The achievement of similar results using the vector C1-C4 and the vector C1-C5 to describe the sorbitol molecule validates their use as geometrical parameters.

It is possible that the association of the sorbitol molecules might produce a shift in the average conformation of the molecule as the result of the interaction, since the sorbitol molecule undergoes infrequent transitions between a family of conformations. However, as can be seen from Figure 7, which plots the conformational probabilities for each of the

torsion angles in the chain, there was no conformational difference found between the bound and unbound sorbitol molecules. A small difference was seen, however, between the average sorbitol conformation in this study and that reported previously.^{22,23} This difference arises only in the terminal torsional angle, and may arise from intermolecular interactions between sugar solute molecules due to the large difference in concentrations between the two studies (infinite dilution in the previous work and 1 molal here), and perhaps the water model used.

Conclusions

The similarity in binding behavior of caffeine with the two sugars glucose and sorbitol implies that the association of sugars with caffeine in an aqueous environment differs somewhat from the self-association of caffeine. Both sugar molecules have hydrophobic groups that structure adjacent water molecules “uncomfortably” in the fashion of a small solute, but this effect is not enough to overcome their strong interactions with water via hydrogen bonding and thus does not cause them to aggregate in solution. However, they can also associate with the hydrophobic faces of caffeine, not only liberating their own structured waters, but also freeing the structured water molecules over the face of the caffeine, which recover their lost hydrogen bond in the process, providing a greater driving force for the association.

This view raises questions about the possibility that caffeine may interact with many other molecular species in green/roasted coffee beans, as well as in brewed coffee. Indeed, low and high molecular weight carbohydrates are important constituents of coffee which, during roasting, undergo degradation processes in terms of decreasing molecular weight and increasing hydrophobicity because of the loss of structural water. It is foreseen, therefore, that the interaction of caffeine with other molecules could increase after roasting. Model carbohydrate structures and their degradation products interacting with caffeine therefore should be investigated for any potential roles under physiological conditions either in taste or in functional properties.

Acknowledgments

This project was supported by a grant from the National Institutes of Health (GM63018). L.T. is the recipient of a fellowship from the Italian Ministero dell'Istruzione, Università e della Ricerca (MIUR, Rome). Support for computational work by CINECA ISCRA grants (Bologna, Italy) is gratefully acknowledged.

References

1. Nehlig A, Daval JL, Debry G. Brain Res Rev. 1992; 17:139–170. [PubMed: 1356551]
2. Cesàro A, Russo E, Crescenzi V. J Phys Chem. 1976; 80:335–339.
3. Lilley TH, Linsdell H, Maestre A. J Chem Soc Faraday Trans. 1992; 88:2865–2870.
4. Stoesser PR, Gill SJ. J Phys Chem. 1967; 71:564–567. [PubMed: 6044492]
5. Chandler D. Nature. 2005; 437:640–647. [PubMed: 16193038]
6. Horman I, Viani R. J Food Sci. 1972; 37:925.
7. D'Amelio N, Fontanive L, Uggeri F, Suggi-Liverani F, Navarini L. Food Biophysics. 2009; 4:321–330.
8. Tavagnacco L, Engström O, Schnupf U, Saboungi ML, Himmel M, Widmalm G, Cesàro A, Brady JW. J Phys Chem B. 2012; 116:11701–11711. [PubMed: 22897449]
9. Tavagnacco L, Schnupf U, Mason PE, Saboungi ML, Cesàro A, Brady JW. J Phys Chem B. 2011; 115:10957–10966. [PubMed: 21812485]
10. Lee CY, McCammon JA, Rossky PJ. J Chem Phys. 1984; 80:4448–4455.
11. Stillinger FH. Science. 1980; 209:451–457. [PubMed: 17831355]
12. Ashbaugh HS, Paulaitis ME. J Am Chem Soc. 2001; 123:10721–10728. [PubMed: 11674005]

13. Huang DM, Chandler D. *J Phys Chem B*. 2002; 106:2047–2053.
14. Liu P, Huang X, Zhou R, Berne BJ. *Nature*. 2005; 437:159–162. [PubMed: 16136146]
15. Zangi R, Berne BJ. *J Phys Chem B*. 2008; 112:8634–8644. [PubMed: 18582012]
16. Shallenberger, RS. *Advanced Sugar Chemistry: Principles of Sugar Stereochemistry*. AVI Publishing Company, Inc; Westport, Connecticut: 1982.
17. Stoddart, JF. *Stereochemistry of Carbohydrates*. Wiley-Interscience; New York: 1971.
18. Bergenstråhle M, Wohler J, Himmel ME, Brady JW. *Carbohydr Res*. 2010; 345:2060–2066. [PubMed: 20705283]
19. Gray MC, Converse AO, Wyman CE. *Appl Biochem Biotechnol*. 2003; 105–108:179–193.
20. Zhang Y, Cremer PS. *Annual Review of Physical Chemistry*. 2009; 61:63–83.
21. Rukiah M, Lefebvre J, Hernandez O, Van Beek W, Serpelloni M. *J Appl Cryst*. 2004; 37:766–772.
22. Lerbret A, Mason PE, Venable RM, Cesàro A, Saboungi ML, Pastor RW, Brady JW. *Carbohydr Res*. 2009; 344:2229–2235. [PubMed: 19744646]
23. Hatcher ER, Guvench O, MacKerell AD. *J Chem Theory Comput*. 2009; 5:1315–1327. [PubMed: 20160980]
24. Brooks BR, Bruccoleri RE, Olafson BD, Swaminathan S, Karplus M. *J Comput Chem*. 1983; 4:187–217.
25. Brooks BR, Brooks CL, MacKerell ADJ, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, et al. *J Comput Chem*. 2009; 30:1545–1614. [PubMed: 19444816]
26. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. *J Chem Phys*. 1983; 79:926–935.
27. van Gunsteren WF, Berendsen HJC. *Mol Phys*. 1977; 34:1311–1327.
28. Darden T, York D, Pedersen L. *J Chem Phys*. 1993; 98:10089–10092.
29. Mason PE, Lerbret A, Saboungi ML, Neilson GW, Dempsey CE, Brady JW. *Proteins*. 2011; 79:2224–2232. [PubMed: 21574187]

Figure 1.

The molecular structure of sorbitol in the fully extended conformation (left), and the molecular structure of caffeine (right), with its dipole moment vector shown in red. The atomic labeling employed in the present study is indicated.

Figure 2.

Contour density maps calculated for the sorbitol atoms and displayed around the caffeine molecule. The illustrated contours enclose the regions with atomic densities 3.5 times the bulk density. The orange contours are for the atoms H11, H12, H2, H3, H4, H5, H61, and H62; the red contours are for the carbon atoms; the green contours are for the sugar oxygen atoms; and the yellow contours are for the hydroxyl protons HO1, HO2, HO3, HO4, HO5, and HO6.

Figure 3. Contours of sorbitol hydroxyl proton atom density relative to the caffeine solute, seen from “above”. The yellow contour encloses the volume with a proton atom density 3.5 times the bulk value.

Figure 4.
A representative “snapshot” from the trajectory of sorbitol molecules binding to the caffeine molecule.

Figure 5.

a) Evolution of the distance between the C1 and Cn atom positions (with $n = 4, 5$, and 6) of the sorbitol molecules interacting with the caffeine molecule. b) Probability of the distance between the C1 and Cn atom positions of the sorbitol molecules interacting ("bound") with the caffeine molecule (black) and the sorbitol molecules "free" in the bulk solution (red). The definition of "bound" and "free" sorbitol molecules is described in the text. The dotted lines display the probability calculated for the atoms C1-C4, the dashed lines for the atoms C1-C5, and the solid lines for the atoms C1-C6.

Figure 6. Probability of the cosine of the angle between the dipole moment vector of the caffeine molecule and the vector given by the C1 C4 atom positions (black) and C1 C5 atom positions (red) of the sorbitol molecules interacting with the caffeine molecule.

Figure 7.

The distribution of the dihedral angles ϕ_1 (H12 C1 C2 H2), ϕ_2 (H2 C2 C3 H3), ϕ_3 (H3 C3 C4 H4), ϕ_4 (H4 C4 C5 H5), ϕ_5 (H5 C5 C6 H6) averaged over the simulation. Shown in red are the distributions calculated for the sorbitol molecules in the bulk solution ("free") whereas in black are displayed the distribution of the sorbitol molecules interacting with the caffeine molecule ("bound").

Table 1

The averaged number of hydrogen bonds between the sorbitol functional groups and caffeine acceptor atoms in the caffeine averaged over the simulation.

Caffeine acceptors	Water	Sorbitol	Sorbitol donors					
			HO1	HO2	HO3	HO4	HO5	HO6
O6	0.971	0.0746	0.0149	0.0154	0.00942	0.00981	0.0117	0.0133
O2	0.968	0.0608	0.0115	0.0117	0.00721	0.00802	0.00929	0.0131
N9	0.988	0.109	0.0194	0.0211	0.0146	0.0196	0.0164	0.0179

Table 2

Computed sorbitol-caffeine binding energies as a function of the density contour level used to define the binding site.

Density cutoff atoms/Å ⁻³	ΔG J · mol ⁻¹
0.02	-5289
0.025	-5586
0.03	-5850
0.035	-6068
0.04	-6318