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Localization and quantification of hydrophobicity: The molecular free energy density (MolFESD) concept and its application to sweetness recognition

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Summary

A method for the localization, the quantification, and the analysis of hydrophobicity of a molecule or a molecular fragment is presented. It is shown that the free energy of solvation for a molecule or the transfer free energy from one solvent to another can be represented by a surface integral of a scalar quantity, the molecular free energy surface density (MolFESD), over the solvent accessible surface of that molecule. This MolFESD concept is based on a model approach where the solvent molecules are considered to be small in comparison to the solute molecule, and the solvent can be represented by a continuous medium with a given dielectric constant. The transfer energy surface density for a 1-octanol/water system is empirically determined employing a set of atomic increment contributions and distance dependent membership functions measuring the contribution of the increments to the surface value of the MolFESD. The MolFESD concept can be well used for the quantification of the purely hydrophobic contribution to the binding constants of molecule-receptor complexes. This is demonstrated with the sweeteners sucrose and sucralose and various halogen derivatives. Therein the relative sweetness, which is assumed to be proportional to the binding constant, nicely correlates to the surface integral over the positive, hydrophobic part of the MolFESD, indicating that the sweetness receptor can be characterized by a highly flexible hydrophobic pocket instead of a localized binding site.

Introduction

Many concepts in chemistry are based on vaguely defined quantities. Nevertheless, they often work surprisingly well for the interpretation of experimental results and the systematic planning of new experiments. A prominent example for this statement is the concept of hydrophobicity. The interaction of a nonpolar molecule with an aqueous solvent or the quasi-attractive interaction of organic compounds or nonpolar groups in the water phase is known as the *hydrophobic hydration* or *hydrophobic interaction*, respectively [1, 2]. Hydrophobic effects play a key role in many chemical phenomena in aqueous solution. The stability of biological membranes as well as the tertiary structure of proteins in aqueous solution depend critically on interactions between nonpolar moieties. In molecular recognition, hydrophobic bonding is one of the most important forces that arise from the interaction of nonpolar regions of a guest molecule (a pharmaceutical drug for example) with its host molecule (the receptor). Hydrophobic effects are also important for various solution phenomena such as surfactant aggregation, mineral flotation, coagulation, complexation, and detergency.

From the point of view of phenomenological thermodynamics, the hydrophobic effect can be quantified using the solvation free energy of a certain molecu-

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lar compound ΔG or the Gibbs transfer free energy change $\Delta\Delta G = \Delta G_{\text{transfer}}$ of this compound between an aqueous and an apolar liquid phase. A similar approach can be used to study the hydrophobic interaction of two molecules in aqueous solution. There are a lot of thermodynamic data available now for a rich variety of molecules including values for the heat of solvation, dissociation and association constants, partition coefficients of molecules in liquid-liquid systems, specific heat capacities, etc. In many cases these quantities have been measured as functions of temperature and other thermodynamic variables over a wide range. However, up to now, there is still no physical model available which allows a clear correlation between microscopic molecular properties (like those which can be measured from isolated molecule experiments or quantum chemical calculations) and macroscopic (thermodynamic) data which are connected to the term hydrophobicity or hydrophobic interaction. For an extensive discussion of this topic see the excellent review of Blokzijl and Engberts [1].

The understanding of the molecular nature behind the hydrophobic effect or at least a uniquely defined molecular scenario which allows correlations of the kind mentioned above is obviously essential for a quantitative treatment of this phenomenon. The aim of the present work is directed towards the establishment of a semi-empirical scenario in which molecular properties or properties of molecular fragments can be directly correlated to partial ΔG values, i.e. correlated to the contribution of hydrophobic interaction to the overall binding constant of a molecular complex (a substrate receptor complex for example). The strategy is to develop a concept which has a clear physical basis on one hand but wherein certain open parameters are fitted to experimental (thermodynamic) data on the other hand. The procedure should be fast enough to be used within QSAR studies or even database screening.

A standard thermodynamic measure for a quantitative comparison of the overall hydrophobicity or lipophilicity of different molecules can be obtained from the partition coefficients P [3–9] or the hydrophobic index logP [10, 11] of the sample in polar-apolar heterogeneous reference systems. The two phases are in equilibrium if the relation

$$\log P = -\frac{\Delta\bar{G}_{\text{I, II}}}{2.303\,RT} = -\frac{\Delta\bar{G}_{\text{transfer}}}{2.303\,RT} \qquad (1)$$

holds. The employment of the partition coefficient in QSAR studies was recently reviewed by Buchwald and Bodor [12].

Fujita et al. [3] could prove within a comprehensive study the additive-constitutive nature of the partition coefficient. Based on their results the hydrophobicity of a molecule – measured, for example, by its hydrophobicity index (log P-value) – can be regarded as the sum of increments related to the hydrophobicities of its fragments, f_i:

$$\log P = \sum_i f_i. \qquad (2)$$

Many authors [4–11, 13–20] published sets of fragmental values for partition coefficients (dominantly for the 1-octanol/water system). In place of these we refer to an empirical quantification of lipophilicity contributions of individual atoms which has been accomplished by Ghose and Crippen [8, 9, 20]. In their treatment the atomic contributions were defined by using a classification of each atomic fragment according to the number and nature of their next (and second next) connected neighbours. Up to now roughly 120 constitutive atom types are listed, describing the atoms in their individual structural environment [8–11, 20]. A modification and extension of this set has been elaborated in the group of the present authors [21]. The subdivision of molecular lipophilicity into fragmental contributions seems to be a prerequisite for the manifestation of local hydrophobicity, however, this scheme is not sufficient in any case. So the volume of the solute molecule, its surface area or its 3D-structure or shape can only be rudimentarily considered in such a scenario.

In a recent paper from the group of the author [22] a molecular hydrophobicity mapping (MHM) approach was suggested which was particularly designed for projecting atomic increment values on the molecular surface (a solvent accessible surface (SAS) or contact surface [23] of a molecule)[1]. The MHM approach is well suited for a qualitative discussion of local hydrophobicity on the basis of incremental contributions because the formalism induces a weighted projection of the incremental f_i-values of Crippen and co-workers [8, 9] onto the molecular surface and so allows an easy visualisation of this quantity. One cannot compute quantitative data like $\Delta G_{\text{transfer}}$ or the partition coefficient P from molecular hydrophobicity maps regardless of the qualitative value of the MHM. Such a quantification can also not be performed on

[1] Here and in the following text we will use the term 'molecular surface' in the sense of Connolly [23], i.e., a contact surface of 'spherical' water probe (radius $r = 1.4$ Å) with a CPK-model of a molecule of given conformation is taken as a reference.

the basis of so called *molecular lipophilicity potentials* (MLP) which have been first introduced by Audry et al. [24] and modified by a variety of authors [25–28]. Again, despite the success of the MLP approach in CoMFA [29] and QSAR [30] there is no physical justification for the use of one or the other distance function in this 'potential' while using fragmental parameters as 'partial charges' located at the positions of the atoms in a molecule [22].

The quantitative treatments in this paper are based on the molecular surface concept. It has been shown [31] that there is a correlation between ΔG, ΔH and $T\Delta S$ (Δ stands for the thermodynamic process of solvation) for many apolar gases solved in water and in n-hexane and the molecular surface area. The Gibbs energies for the transfer of apolar solutes from nonpolar solvents to water have also been correlated with accessible molecular surface areas and it was shown [32–44] that the Gibbs energy per surface area involved in the transfer of apolar compounds from a hydrocarbon-like solvent to water amounts to 70 to 130 $\mathrm{J\,mol^{-1}\ A^{-2}}$. The exact magnitude strongly depends on the reference solvent, the computational procedure used to determine the surface area, and the fitting strategy. Abraham [36, 37] as well as Richards and co-workers [42, 43] assumed that the transfer free energy $\Delta G_{\mathrm{transfer}}$ for one mole substance from one solvent to the other can be composed of additive contributions which are related to the solvent accessible surface of fragments of the molecule. A similar scenario has been already suggested by Tanford [35]. The surface concept was also used in theoretical works like that of Lee [45] whose considerations were based on the assumption that the change of enthalpy and entropy are linear functions of the surface area of the solute. A promising ab initio based approach to localize the electrostatic part of solvation free energy on molecular surface area has recently been presented by Luque and co-workers [48]. Kellogg and Abraham [46, 47] used empirical atom-based hydrophobic parameters to estimate protein-ligand binding constants. The HINT program has been proved to be useful in QSAR and CoMFA studies.

Independently from the fact that the molecular surface concept is very useful for the quantitative treatment of thermodynamic data related to the solvation process or the transfer of a solute molecule from one solvent to another, it should be noticed that these data are in general not in quantitative agreement with the macroscopic surface tension or other surface energies [49].

In this work we present a concept wherein the thermodynamic data ΔG, ΔH and $T\Delta S$ of solvation or transfer can be calculated as a surface integral over a quantity which has been termed *molecular free energy surface density* (MolFESD) $\rho(\mathbf{r})$, a continuous function of the surface coordinates $\mathbf{r}$ which can be considered as the free energy per unit surface of the solute. The paper is organized as follows: In the next section it is shown analytically that the enthalpic part of $\rho(\mathbf{r})$ can be obtained from electrostatic argument for those cases where the solute molecule is modelled as a charge distribution separated from a continuous dielectric solvent. Some arguments are reviewed for the assumption that $T\Delta S$ can be modelled as a surface extensive property. This is followed by a section that deals with the explicit construction of the model. It is outlined how to compose the MolFESD from atomic fragments of the solute molecule. The parameterisation is based on the partition of solute molecules in an 1-octanol/water system. We have chosen this system because there is a broad spectrum of experimental data available. It is most relevant for the hydrophobic interaction in biological systems since it has been demonstrated that hydrated 1-octanol can well serve as a hydrophobic biophase analogue [6, 50, 51]. In the application section we demonstrate the quantification of hydrophobic interactions as a measure for the sweet taste sensation of various compounds using the MolFESD approach. The final section contains a summary and an outlook to further applications.

Free energy of solvation as a function of the solvent exposed surface

In this section the physical basis for the introduction of the MolFESD concept is given and some limitations are outlined. We discuss the evaluation of the electrostatic interaction energy between solute and the surrounding solvent as well as the entropic contributions in terms of a surface energy density functional.

Considering solution thermodynamics of different classes of molecules, there are two limiting cases of (i) ionic/strongly polar solutes and (ii) nonpolar organic compounds. While the free energy of solvation, ΔG_{solv}, of the former tends to be dominated by electrostatic energy, it is the work of cavity formation that makes the dominant contribution to ΔG_{solv} of the latter. In the following we discuss how the molecular solvation energy in whatever case can be calculated as a function of the solvent-exposed molecular surface.

Ionic and strong polar solutes

Classical electrostatics provide an important tool for the calculation of solvation energies of polar and ionic solutes in aqueous solution. In the widely accepted macroscopic continuum model [52–64] the structure of solvent media is neglected. Continuum solvent models have been developed for use with both quantum mechanical and empirical force fields [65–76]. While taking an explicit representation of solute atoms – e.g. an arbitrary charge distribution in a cavity – the average electrostatic properties of a solvent medium are represented by an infinite polarizable dielectric medium with constant permittivity ε_ν. Accounting for atomic polarizability, the molecular cavity is defined as a low dielectric medium $\varepsilon_\mu \ll \varepsilon_\nu$.

By extrapolating thermodynamic properties of macroscopic systems to a microscopic level, electrostatic contributions to free energies of solvation (ΔG_e) can be calculated in terms of the difference in the total reaction field energy of solvent and vacuum upon the cavity-bound charge distribution. Electrostatic potential maps are obtained by solving the Poisson equation, which can either be done numerically [53–56, 59, 61–65] or analytically [77–81] for simple geometries. ΔG_e is derived by an integration or summation procedure of the electrostatic energy densities of a hypothetical solute in vacuum and in solution over all space.

An alternative two-step approach for ΔG_e is offered by calculating the transfer energy ΔG_τ [82, 83] for the substitution of a homogeneous and infinite dielectric medium with permittivity ε_μ with a dielectric medium ε outside the molecular cavity. ε stands either for vacuum or solvent medium. ΔG_τ can be calculated from the gradients of the electrostatic potentials $\nabla\varphi_\mu$ (homogeneous dielectric medium) and $\nabla\varphi_\eta$ (dielectric boundary at the cavity surface):

$$\Delta G_\tau = \tfrac{1}{2}\int_V [(\varepsilon_\mu - \varepsilon)\nabla\varphi_\mu\nabla\varphi_\eta]\,\mathrm{d}V, \tag{3}$$

V is the volume of the substituted dielectric body. ΔG_e is a sum of two parts: (i) Transfer energy of a medium ε_μ into the vacuum outside the molecular cavity and (ii) transfer energy of a medium ε_ν into a system of fixed charges embedded in a homogeneous medium with permittivity ε_μ. Following the continuum model solute molecules are approximated by fixed charge distributions embedded in a dielectric cavity with permittivity ε_m (Figure 1).

Using Equation 3, the solvation energy of a solute molecule is given by integration over the volume occupied by the solvent:

$$\begin{aligned}\Delta G_e = \ \Delta G_{\tau^{\mathrm{I}}-\tau^{\mathrm{II}}} = \tfrac{1}{2}\int_V [&(\varepsilon_\mu - \varepsilon_\nu)\nabla\varphi_\mu\nabla\varphi_\nu \\ &+(1-\varepsilon_\mu)\nabla\varphi_\mu\nabla\varphi_0]\,\mathrm{d}V,\end{aligned} \tag{4}$$

where

$$\nabla\varphi_1\nabla\varphi_2 = \nabla(\varphi_1\nabla\varphi_2) + \varphi_1\nabla^2\varphi_2. \tag{5}$$

The indices μ, ν and 0 represent solute, solvent, and vacuum, respectively. If the ionic strength of the solvent is negligible, Laplace's equation $\nabla^2\varphi = 0$ is valid outside of the cavity, where no charges are present:

$$\begin{aligned}\Delta G_e = \ \tfrac{1}{2}\int_V [&\varepsilon_\mu\nabla(\varphi_\nu\nabla\varphi_\mu) - \varepsilon_\nu\nabla(\varphi_\mu\nabla\varphi_\nu) \\ &+\nabla(\varphi_\mu\nabla\varphi_0) - \varepsilon_\mu\nabla(\varphi_0\nabla\varphi_\mu)]\,\mathrm{d}V.\end{aligned} \tag{6}$$

By application of the Gauss theorem

$$\int_V \nabla(\varphi_1\nabla\varphi_2)\,\mathrm{d}V = \oint_S (\varphi_1\nabla\varphi_2\cdot\mathbf{n}_{\mathrm{surf}})\,\mathrm{d}S, \tag{7}$$

Equation 6 is transformed into a surface-integral over the closed surfaces S_{tot} of the solvent along the cavity S_c and at the outer boundery of the solvent S_b.

$$\begin{aligned}\Delta G_e = \ \tfrac{1}{2}\oint_{S_{\mathrm{tot}}} \{&[(\varepsilon_\mu\varphi_\nu - \varepsilon_\mu\varphi_0)\nabla\varphi_\mu - \varepsilon_\nu\varphi_\mu\nabla\varphi_\nu \\ &+\varphi_\mu\nabla\varphi_0]\cdot\mathbf{n}_{\mathrm{surf}}\}\,\mathrm{d}S\end{aligned} \tag{8}$$

with $S_{\mathrm{tot}} = S_c + S_b$.

The terms in rectangular brackets correspond to a scalar energy density functional, which is only nonzero onto the boundaries of the solvent. Since

$$\lim_{r_S\to\infty}\oint_{S_b} (\varphi_1\nabla\varphi_2\cdot\mathbf{n}_{\mathrm{surf}})\,\mathrm{d}S = 0, \tag{9}$$

for very dilute solutions ΔG_e is mainly a function of the cavity surface within the restrictions of this model.

Nonpolar solutes

Experimental measurements related to the hydrophobic effect are based either on the analysis of free energies, enthalpies, and entropies of solvation or the corresponding thermodynamic changes of these quantities for the transfer process from a nonaqueous solvent to water [6, 31, 36, 37, 84–86]. For several nonpolar compounds thermodynamic measurements of the hydrophobic hydration at $T = 289$ K result in a positive free energy due to a significant decrease

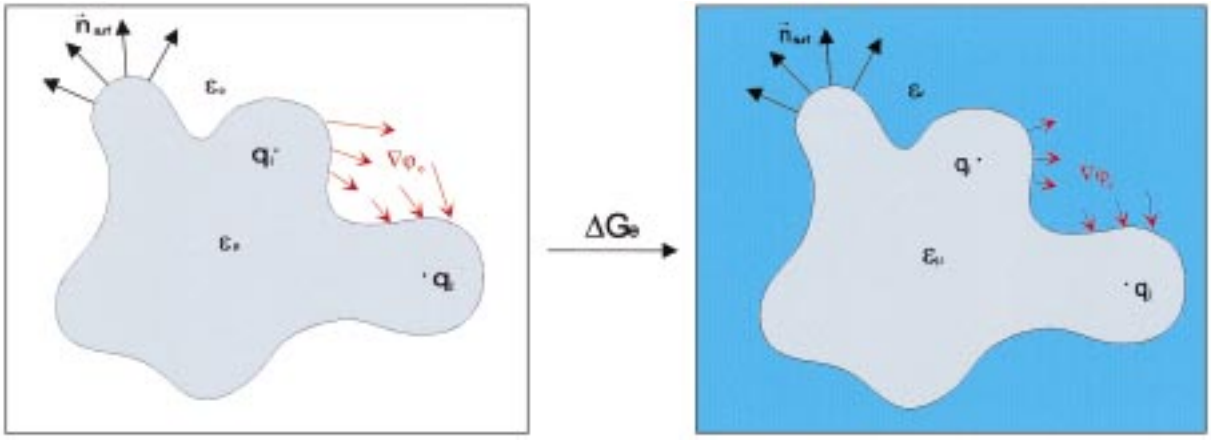

Figure 1. A dielectric continuum representation of a molecular scenario $q_i \cdots q_j$, ε_μ, which is transferred from vacuum, ε_0 (left-hand side) to a continuous solvent, ε_ν. The dielectric solvent weakens the normal components $\mathbf{n}_{\text{surf}} \cdot \nabla\varphi$ of the electrostatic field and thus the total field (red arrows) at the molecular surface.

of entropy during the solvation process in water, i.e., one finds $\Delta G_{\text{hyd}} > 0$, $\Delta H_{\text{hyd}} < 0$ (but small), and $T\Delta S_{\text{hyd}} < 0$. For the transfer of nonpolar molecules from an organic solvent to water experimental results show that the enthalpy change contributes dominantly to the total free energy change, i.e., $\Delta G_{\text{hyd}} > 0$, $\Delta H_{\text{hyd}} > 0$, and $T\Delta S_{\text{hyd}} < 0$ (but small). Abraham et al. [36, 37, 85, 86] constructed a general set of parameters for the solution of gaseous nonelectrolytes in various solvents in order to reproduce free energies, enthalpies, and entropies by the use of simple linear relationships. They used parameters closely correlated to the volume of the solute and to the molecular surface. A correlation between thermodynamic solvation data and molecular surfaces was also established by Privalov et al. [31]. All these results indicate that the molecular surface concept is reasonable when discussing the thermodynamic data, in particular the entropy, in a quantitative manner. However, these empirical findings cannot serve as a physical basis for a semiempirical model.

There has been a considerable effort in the last decades in order to establish a theoretical concept, particularly for the entropic part of the problem. We first refer to the old concept of the *eisberg* model proposed by Frank and Evans [87]. In this model the decrease of entropy in the hydrophobic hydration is explained with the occurrence of a clathrate-like hydration shell structure, i.e. the intermolecular hydrogen bond network between water molecules in the first hydration shell leads to a significantly lower degree of freedom in comparison with bulk water in this scenario. It is clear that this entropy contribution can be roughly set proportional to the solvent accessible surface (SAS) of the solute molecule. However, it has been demonstrated [88–90] that the iceberg model is too simple to explain the experimental findings. The decrease of entropy measured at hydrophobic hydration can be rather related to an excluded volume effect while structural effects in the hydration shell tend to increase the solubility in water. An interesting attempt to explain thermodynamic data of solvation of apolar compounds in liquid water is the scaled particle theory (SPT) in which all particles are assumed to be spherical with diameters defined such that thermodynamic quantities of solution and pure liquid are reproduced. Pierotti applied this theory to study hydrophobic effects [91–93] and separated the dissolution of a particle into two steps: first, the creation of a cavity in the solvent which has the appropriate size to accommodate the solute molecule and second, the onset of the interactions between the solute and the solvent. The cavity formation has a basis which is dominantly entropic in nature. It was found that the free energy of cavity formation ΔG_c is roughly proportional to the number of solvent molecules in the first solvation shell [94] (Figure 2). For the case where the size of the solute is considerably bigger than that of the solvent molecules the solvation number can be set proportional to the total area of the SAS, i.e., the contribution $T\Delta S_c$ can be represented as a surface integral as well.

In a recent theoretical paper of Ben-Naim et al. [95] on the solvation free energy of a nonpolar hard sphere system a simple relation (Equation 10) was established, i.e., in addition to a constant term the free

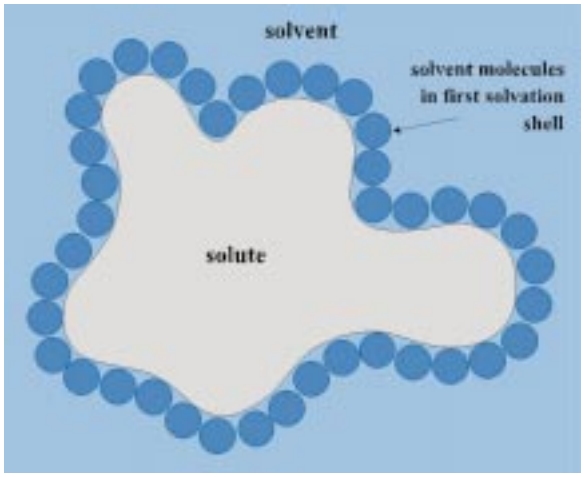

Figure 2. Schematic representation of a solute with its first solvation shell.

energy difference becomes proportional to the surface and the volume of the solute:

$$\Delta G_n \cong c_0 + c_2 R^2 + c_3 R^3. \tag{10}$$

Our further considerations are based on this concept. If the diameter of the cavity is significantly larger than the solvent diameter, ΔG_n is essentially dominated by the latter terms in Equation 10. We note that the volume V of an arbitrarily shaped object can be written as a surface integral using the divergence theorem [96]

$$V = \int_V \mathrm{d}V = \tfrac{1}{3} \oint_S \mathbf{R} \cdot \mathbf{n}_{\mathrm{surf}} \, \mathrm{d}S. \tag{11}$$

If we neglect the constant term in Equation 10 and apply Equation 11 to the volume dependent term of Equation 10, we obtain a surface integral representation of the free energy of cavity formation

$$\Delta G_n \cong \oint_S F(\mathbf{R}_S) \, \mathrm{d}S \tag{12}$$

with a properly chosen function F of the surface points $\mathbf{R}_S$.

Since purely electrostatic as well as nonpolar terms of the free energy of solvation can be represented by a surface integral, we postulate such a relation to be valid for all intermediate cases:

$$\Delta G_{\mathrm{solv}} = \Delta G_e + \Delta G_n = \int_S \rho(\mathbf{r}_S) \, \mathrm{d}S \tag{13}$$

wherein $\rho(\mathbf{r}_S)$ is a suitably chosen function of the surface coordinates $\mathbf{r}_S$.

The empirical molecular free energy surface density (MolFESD) concept

Based on the argumentation presented in the previous section we formulate an empirical model for the transfer free energy $\Delta\Delta G = \Delta G_{\mathrm{transfer}}$ for a substance from aqueous solution to a 1-octanol solution. The experimental data used for the parametrization are taken from partition coefficients P of the substance in a 1-octanol/water system, i.e., strictly speaking we have two liquid phases which are saturated solutions. It has been argued [6, 50, 51, 97, 98] that the octanol phase can be adequately considered as a hydrophobic biophase analogue. This argument will become important in what follows below.

We started from the assumption of Richards et al. [33, 42] that the transfer free energy $\Delta G_{\mathrm{transfer}}$ for one mole of substance from one solvent to the other can be composed of additive contributions of fragments which are related to the solvent accessible surface of the solute.

$$\Delta G_{\mathrm{transfer}} = -2.303 \, RT \, \log \, P = \sum_i \Delta G_i \tag{14}$$

In our approach the $\Delta G_{\mathrm{transfer}}$-value does not occur as a sum but as a surface integral over a quantity which is termed *molecular free energy surface density* (MolFESD), given the symbol ρ.

$$\Delta G_{\mathrm{transfer}} = \int_S \rho(\mathbf{r}) \, \mathrm{d}S. \tag{15}$$

The quantity $\rho(\mathbf{r})$ measures to what extent a certain surface element contributes to the $\Delta G_{\mathrm{transfer}}$-value per unit surface. This notion is similar to the summation of discrete surface elements in the work of Luque et al. [48]. The MolFESD value has a physical meaning only on the molecular surface. It corresponds to the surface free energy per unit area in macroscopic liquid/liquid two phase systems or to the surface tension in liquid/gas systems. Insofar our approach is an extrapolation of thermodynamic concepts to a molecular scale.

Similar to Equation 14, the MolFESD can be represented as a superposition of local contributions

$$\rho(\mathbf{r}_s) = \sum_i \rho_i(\mathbf{r}_s). \tag{16}$$

On the basis of this representation the incremental contributions to the total free energy (Equation 14) follow as

$$\Delta G_i = \int_S \rho_i(\mathbf{r}_s) \, \mathrm{d}S. \tag{17}$$

The summation in Equation 16 is taken over atomic or molecular increments i. The incremental contributions ρ_i are chosen in a similar way as the mapping functions considered earlier [22]. Only atomic increments were considered and it was assumed that ρ_i can be adequately modelled by

$$\rho_i(\mathbf{r}_s) = F_i \mu_i(\mathbf{r}_s) \tag{18}$$

with an increment specific constant F_i and a membership function μ_i in the sense of fuzzy set theory [99] which measures to what extent a surface point is associated with the increment i. We used the membership function which was introduced earlier [100]:

$$\mu_i(\mathbf{r}_s) = \frac{\exp(-2c_i/\delta_i) + 1}{\exp(2(d_i - c_i)/\delta_i) + 1} \tag{19}$$

with $d_i = |\mathbf{r}_S - \mathbf{r}_i|$ and a normalization

$$\sum_i \mu(d_i;\ c_i,\ \delta_i) = 1. \tag{20}$$

The characteristic proximity parameters c_i and δ_i determine in what way an increment i influences the MolFESD-values at a certain surface point.

The membership function μ has a priori no physical meaning. It fulfils two conditions: It is smooth and has finite values for $r < c$ where c is a cutoff value which is termed the *proximity distance* of an increment. The value of the parameter c should be larger than any van der Waals radius of the increment in the molecule under consideration. For distances $r > c$ the function values of μ rapidly tend towards zero, i.e., the corresponding increment does not significantly contribute to the overall value of the MolFESD. It should be noted here that the individual membership functions implicitly depend on all atomic coordinates of the molecular system as a consequence of the choice of the normalization condition.

The MolFESD function represents a weighted average of all the F_i values of atomic increments i for which $d_i < c_i$ is fulfilled, where d_i is the distance from a surface point to the center of the ith increment. All atoms which are further away from the surface point do not contribute significantly [22, 100]. The quantities μ_i are related to weighting factors p_i for the contributions of individual atomic free energy surface densities F_i to the local MolFESD-value.

$$\rho(\mathbf{r}_S) = \sum_i \frac{\mu(d_i;\ c_i,\ \delta_i)}{\sum_j \mu(d_j;\ c_j,\ \delta_j)} \cdot F_i = \sum_i p_i \cdot F_i. \tag{21}$$

The proximity parameters c_i and δ_i for all atomic increments have been determined in the group of the authors [21]. Similar to Equation 21 the quantity

$$S_i = \int_S p_i(\mathbf{r}_S)\, \mathrm{d}S \tag{22}$$

can be interpreted as the contribution of the increment i to the total molecular surface S, and one simply has

$$S = \sum_j S_i. \tag{23}$$

One should note here that, although the total surface is given as a sum of increment surfaces S_i, the latter are not strictly local properties. A given surface point can only be associated to an increment with a certain weight. There is no sharp borderline between the individual S_i areas, in contrast to the treatment of other authors [42, 43, 48].

Furthermore, the surface increments do depend on the relative positions of all the other increments, so S_i is explicitly dependent on the molecular conformation. The same atomic increment i will, in general, contribute to the total $\Delta G_{\text{transfer}}$-value in a different way, even if the F_i-value of the increment is the same [100]. This fact opens the possibility to include the conformation of a given compound in an empirical parametrization strategy.

Following Equations 14 and 15 the overall hydrophobicity of a molecule (represented by logP data) can be easily calculated. The results are equivalent to those which are generated by the MOLCAD program developed in our group. A representative dataset has been recently studied with several popular computational descriptors of hydrophobicity [101]. In a multivariate analysis on a database of rougly 160 molecules, similar to other surface-based methods like HINT [46, 47], the total LogP values derived by MolFESD proved satisfactory.

The MolFESD concept has further been applied [102] in order to estimate the optimal position and orientation of a solute molecule at the interface region of two liquid phases. The argumentation therein was the following: Let ΔG_{I} and ΔG_{II} be the free energies for dissolving a molecule in solvent I and II, respectively, the transfer free energy $\Delta G_{\text{transfer}}$ is simply given as

$$\Delta G_{\text{transfer}} = \Delta G_{\text{II}} - \Delta G_{\text{I}}. \tag{24}$$

Following the concepts outlined above, the free energy ΔG_i of solvation of a solute molecule in solvent (i) can be described as

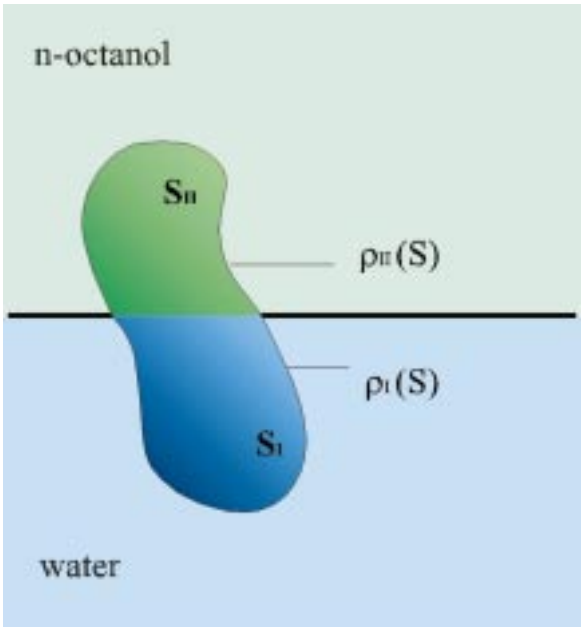

Figure 3. Optimal alignment of a molecule at a phase boundary.

$$\Delta G_i = \int_S \rho_i(S)\, \mathrm{D}S \tag{25}$$

wherein $\rho_i(S)$ is the free energy surface density for the solute molecule in solvent (i). Comparing Equation 24 to Equation 25 one has

$$\rho_T(S) = \rho_{\mathrm{II}}(S) - \rho_{\mathrm{I}}(S). \tag{26}$$

The consequent application of the MolFESD concept to a molecule which is brought from vacuum to a liquid/liquid interface region (Figure 3) leads to

$$\Delta G_{\mathrm{INT}} = \Delta G^{\mathrm{I}}_{\mathrm{INT}} + \Delta G^{\mathrm{II}}_{\mathrm{INT}} \tag{27}$$

with

$$\Delta G^{i}_{\mathrm{INT}} = \int_{S_i} \rho_i(S)\, \mathrm{d}S_i. \tag{28}$$

Using Equation 28 one obtains with $S_{\mathrm{I}} + S_{\mathrm{II}} = S$

$$\Delta G_{\mathrm{INT}} = \int_{S_{\mathrm{I}}} \rho_{\mathrm{I}}(S)\, \mathrm{d}S + \int_{S_{\mathrm{II}}} \rho_{\mathrm{I}}(S)\, \mathrm{d}S + \int_{S_{\mathrm{II}}} \rho_T(S)\, \mathrm{d}S = \Delta G_{\mathrm{I}} + \int_{S_{\mathrm{II}}} \rho_T(S)\, \mathrm{d}S. \tag{29}$$

The first part of this expression does not depend on the position and orientation of the molecule in the surface region while the second does. Obviously ΔG_{INT} becomes a minimum – related to a stable placement of the molecule – when the second term in Equation 29 becomes minimal. This condition was used in order to calculate the immersion depths of various local anaesthetics in a two liquid hydrophobic/hydrophilic interface [102]. A generalization of this concept is used in the next section for quantitative treatment of an induced fit situation of a highly flexible hydrophobic pocket of a receptor to various sweeteners.

Quantification of hydrophobic contributions to sweetness recognition

To our best knowledge the receptor for the recognition of sweeteners is not known up to now. It may happen that there is not only one receptor but several different ones. Therefore, in this paper we only will consider substances which are derivatives of sucrose and it seems to be reasonable to assume that all of them are recognized by the sucrose receptor. The current conception of this receptor model dates back to Shallenberger [103] and Kier [104], who postulated that the sweetness receptor should contain at least three structural components: a proton donor functionality AH, a proton acceptor position B, and a hydrophobic binding site X, all arranged in a triangle conformation – the sweetness triangle. Lichtenthaler and co-workers [105] postulated, on the basis of hydrophobic maps introduced by the group of the present authors [22], that the hydrophobic site should be a rather extended region. A correlation was found between the relative sweetness of several sucrose derivatives and their logP data derived by a Crippen-type approach [106]. However, there has been no attempt to quantify hydrophobic contributions to the free energy of ligand-receptor binding.

In this work we demonstrate that the MolFESD concept is capable of describing a quantitative local representation of hydrophobicity on the SAS of ligand molecules. In particular we show that the surface integral of MolFESD due to that part of the hydrophobic surface of the ligand explicitly interacting with the receptor correlates well with the free energy of binding. Therefore the method can be used to calculate the energetic and (implicitly) entropic contribution of a hydrophobic part of a ligand to the total ligand-receptor interaction.

Our model approach has been developed in two steps (models a and b). The first two assumptions for both models can be summarized as follows:

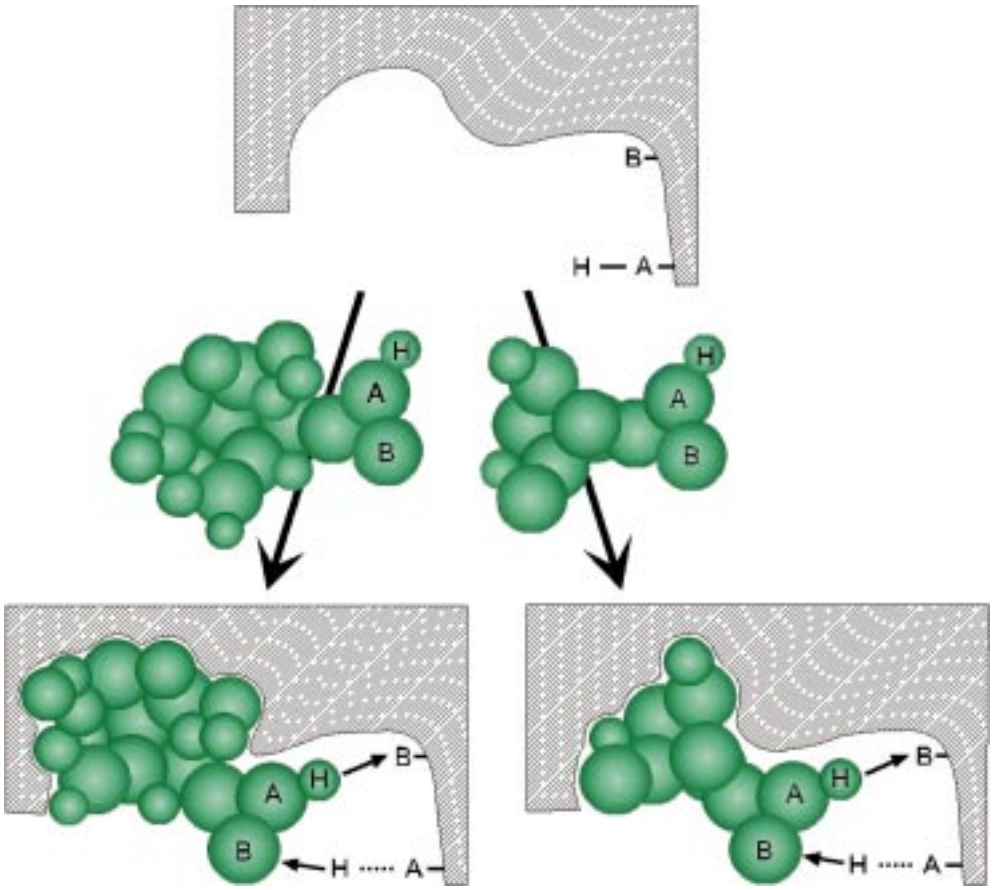

Figure 4. Association of two different molecules to a highly flexible hydrophobic receptor pocket (schematically).

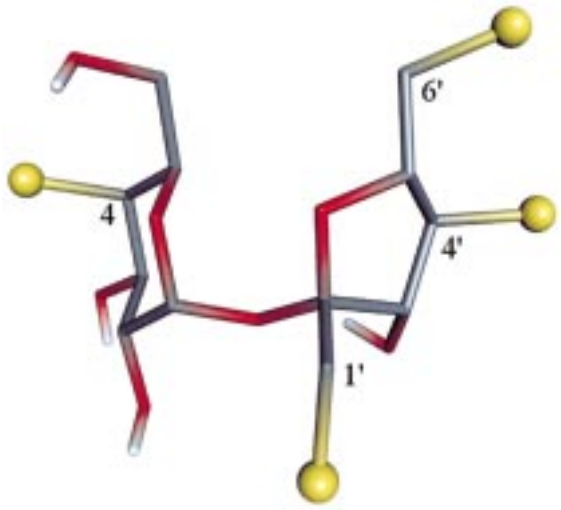

Figure 5. Backbone of the sweetener sucrose – energetically favorable conformation. Substitution positions enhancing sweetness are marked yellow.

(i) Like other authors [107], we suppose that the sweetness is proportional to the equilibrium constant K for the association of a sweetener and its receptor.

(ii) We followed the results of Shallenberger [103], Kier [104] and Lichtenthaler [105] for the sweetness receptor model. In contrast to these authors, however, in our approach the hydrophobic region is highly flexible, i.e., it can cover a hydrophobic part of the sweetener quantitatively without investing conformational free energy.

(iii) We further adopt that a water saturated 1-octanol solution can be considered as a hydrophobic biophase analogue.

(iiia) In model (a) a highly flexible hydrophobic receptor pocket is emulated as stated above (Figure 4). In this case the hydrophobic contribution to the overall ΔG-value for the association of the molecule to the receptor can be calculated in an analogous manner as has been described in the last section, i.e., one has to minimize

Table 1. Relative sweetness and correlation data of sucrose chlorine derivatives calculated from model (a)

	Substitution positions	Surface increment (Å^2)	Surface integral (log P)	ΔG_{INT} (kJ/mol)	Relative sweetness
1	–	3.160	0.001	0.010	1[108]
2	1′-Cl	35.423	0.217	−1.239	20[108]
3	6′-Cl	33.185	0.209	−1.191	20[108]
4	1′-Cl, 4′-Cl	83.789	0.542	−3.091	30[109]
5	1′-Cl, 6′-Cl	76.482	0.549	−2.617	80[110]
6	1′-Cl, 4′-Cl, 6′-Cl	130.135	1.323	−7.549	100[110]

Table 2. Relative sweetness and correlation data calculated from model (a) of halogen derivatives of 4-chloro-deoxy-*galacto*-sucrose

	Substitution positions	Surface increment (Å^2)	Surface integral (log P)	ΔG_{INT} (kJ/mol)	Relative sweetness
7	–	25.185	0.099	−0.562	5[108]
8	6′-Cl	65.902	0.341	−1.946	50[111]
9	1′-Cl	60.527	0.330	−1.884	120[111]
10	4′-Cl, 6′-Cl	121.240	1.075	−6.132	160[111]
11	1′-Cl, 4′-Cl	112.652	0.667	−3.808	220[111]
12	1′-Cl, 6′-Cl	114.549	0.626	−3.574	600[110]
13	1′-6′-Cl, 4′-F	156.320	1.181	−6.736	1000[111]
14	1′-Cl, 4′-Cl, 6′-Cl	169.970	1.531	−8.735	2200[111]
15	1′-Cl, 6′-Cl, 4′-Br	171.891	1.614	−9.210	3000[111]
16	1′-6′-Cl, 4′-I	177.928	2.140	−12.211	3500[111]

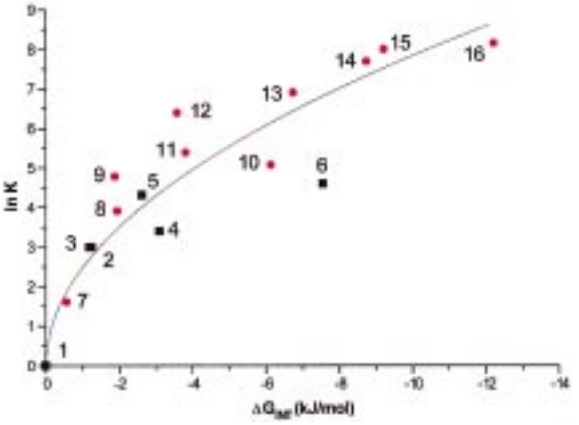

Figure 6. Relative sweetness $\ln K$ as a function of ΔG_{INT} (as calculated on the basis of model (a) for halogen derivatives of sucrose (squares) and 4-chloro-4-deoxy-*galacto*-sucrose (circles). The numbers correspond to Tables 1 and 2.

$$\Delta G_{\mathrm{INT}} = \int_{S_R} \rho_T(S)\,\mathrm{d}S, \tag{30}$$

where S_R is the surface area of hydrophobic interaction between the substrate and the receptor.

Before introducing a refinement of model (a), the results will be discussed. The minimisation of the hydrophobic surface integral was applied to a series of sucrose and 4-chloro-4-deoxy-*galacto*-sucrose halogen derivatives (Tables 1 and 2, Figure 5) to exhibit the relation between the hydrophobic surface and the relative sweetness of the selected derivatives. In this case the ΔG-values should only differ by the hydrophobic contribution ΔG_{INT} which is obviously a minimum value when the integration area in Equation 30 is taken as that one where $\rho_T(S)$ takes a positive value. This is equivalent to the assumption that the receptor will in any case cover all of the hydrophobic part of the molecules under consideration. In Table 1 the calculated data based on model (a) and the corresponding sweetness [107] are shown for some chlorine derivatives of sucrose. In Table 2 the same informa-

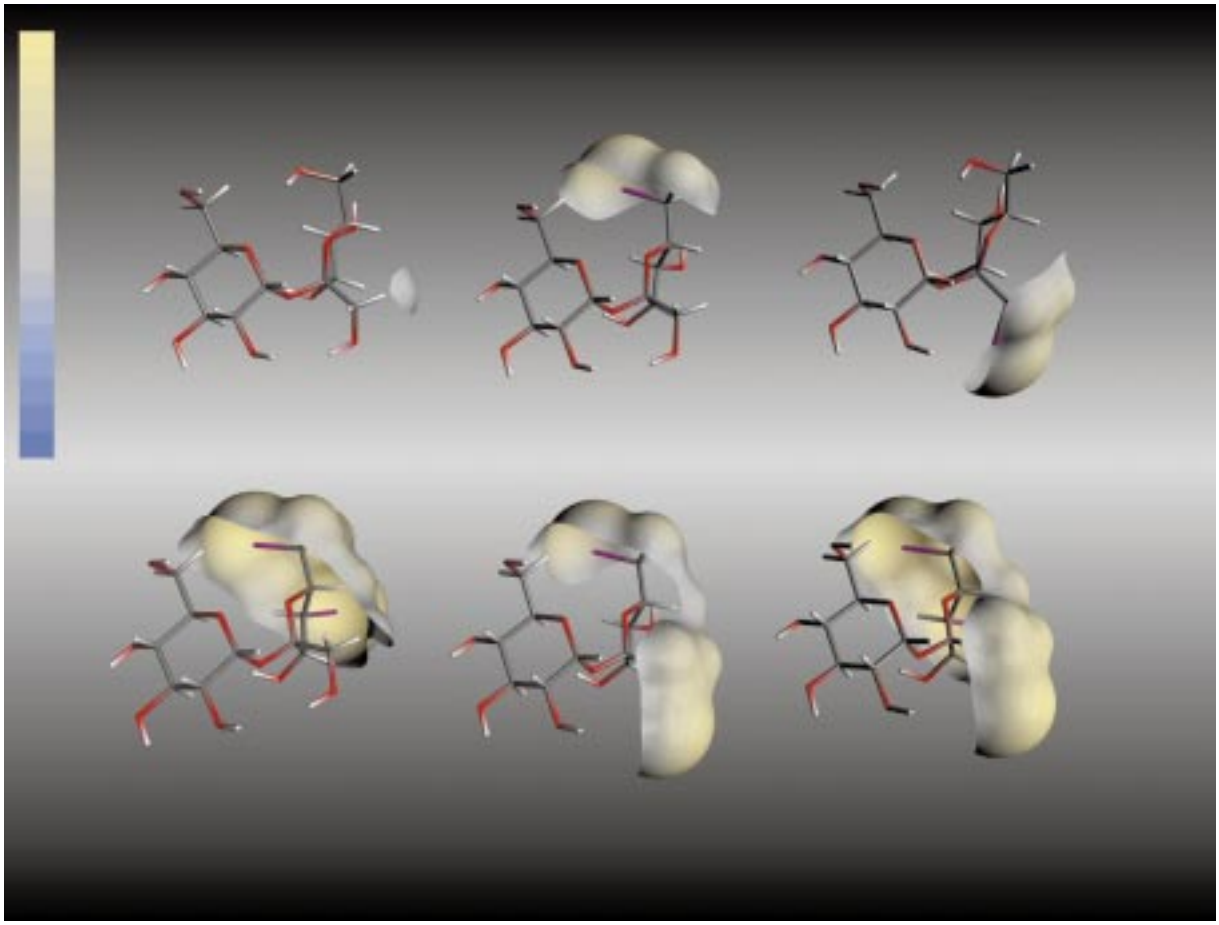

Figure 7. Hydrophobic surface patches with colour coded MolFESD-values (yellow: negative/hydrophobic, blue: positive/hydrophilic) of different chlorine derivatives of sucrose calculated on the basis of model (a). The visualisation was performed with the MOLCAD package [112].

tion is given for the 4-chloro-4-deoxy-*galacto*-sucrose halogen derivatives.

The natural logarithm of the sweetness data (ln K) versus the ΔG_{INT} values corresponding to the hydrophobic surface integral for the sucrose and the 4-chloro-4-deoxy-*galacto*-sucrose derivatives, respectively, are drawn in Figure 6. A correlation is found between ln K and ΔG_{INT} for most of the experimental data but this correlation is obviously non-linear. In particular, the less halogenated molecules show smaller ΔG_{INT} values than those expected from a linear extrapolation of the multiple substituted molecules. This result indicates that the hydrophobic pocket of the receptor is obviously not flexible enough in order to fit only to the hydrophobic parts of the MolFESD, but covers also a certain hydrophilic area where the MolFESD becomes negative.

A refined model (b) was formulated, wherein it was assumed that

(iiib) for all molecules under consideration most of the furanoid ring is covered by a flexible hydrophobic receptor pocket. The integration over the MolFESD has to be done along the surface of the furanoid ring and all remaining hydrophobic parts of the surface.

Based on this assumption the borderline of the integration area covering the fructose portion was defined as the intersection of a plane perpendicular to the C_1–$C_{2'}$ interconnection (see Figure 9). A slight shift of this plane backwards and forwards resulted in a collective ΔG_{INT} shift but did not influence the correlation. Accounting for the hydrophobic interaction of the galactose portion with the receptor, the ΔG_{INT} values of all 4-chloro-4-deoxy-*galacto*-sucrose derivatives were shifted by an additive con-

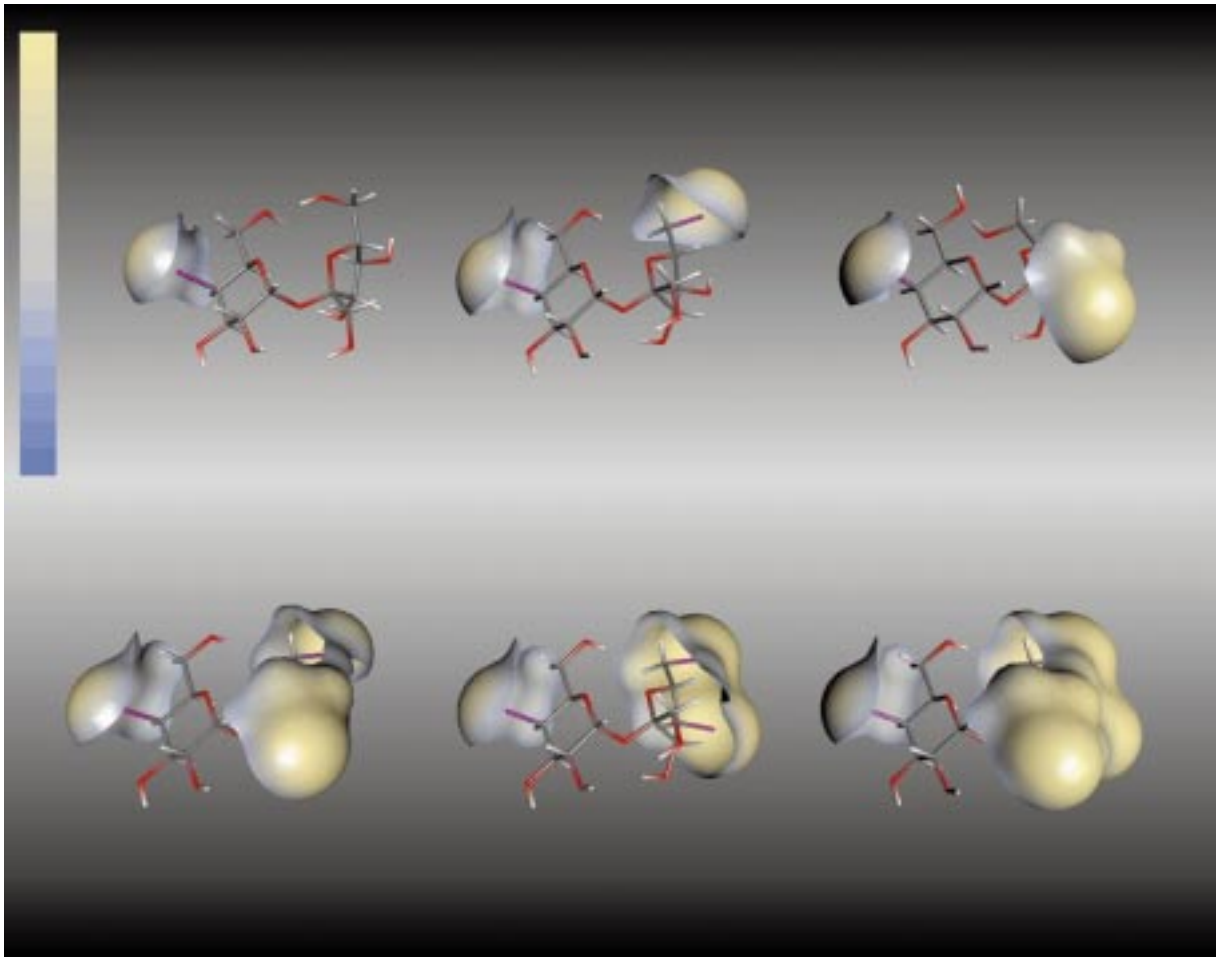

Figure 8. Hydrophobic surface patches with colour coded MolFESD-values (as in Figure 7) of different chlorine derivatives of the 4-chloro-4-deoxy-*galacto*-sucrose calculated on the basis of model (a).

Table 3. Relative sweetness and correlation data from model (b) of chlorine derivatives of sucrose

	Substitution positions	Surface increment (Å^2)	Surface integral (log P)	ΔG_{INT} (kJ/mol)	Relative sweetness
1	–	139.644	−1.730	0	1[108]
2	1′-Cl	163.243	−1.257	−2.7	20[108]
3	6′-Cl	157.286	−0.998	−4.176	20[108]
4	1′-Cl, 4′-Cl	170.018	−0.081	−9.407	30[109]
5	1′-Cl, 6′-Cl	167.468	−0.229	−8.562	80[108]
6	1′-Cl, 4′-Cl, 6′-Cl	179.656	1.060	−15.918	100[111]

Table 4. Relative sweetness and correlation data from model (b) of halogen derivatives of 4-chloro-4-deoxy-*galacto*-sucrose

	Substitution positions	Surface increment (Å^2)	Surface integral (log P)	ΔG_{INT} (kJ/mol)	Relative sweetness
7	–	146.534	−1.793	−6.439	5[108]
8	6′-Cl	157.780	−0.927	−11.379	50[111]
9	1′-Cl	154.785	−0.968	−11.147	120[111]
10	4′-Cl, 6′-Cl	164.871	0.318	−18.487	160[111]
11	1′-Cl, 4′-Cl	166.641	0.07	−16.711	220[111]
12	1′-Cl, 6′-Cl	168.201	−0.146	−15.836	600[110]
13	1′-6′-Cl, 4′-F	163.861	0.817	−21.334	1000[111]
14	1′-Cl, 4′-Cl, 6′-Cl	174.595	1.182	−23.414	2200[111]
15	1′-Cl, 6′-Cl, 4′-Br	178.468	1.258	−23.851	3000[111]
16	1′-6′-Cl, 4′-I	180.131	1.828	−27.101	3500[111]

Figure 9. The integration area covering the five-membered ring of sucrose is given by the intersection of a plane perpendicular to the C_1–$C_{2'}$ interconnection.

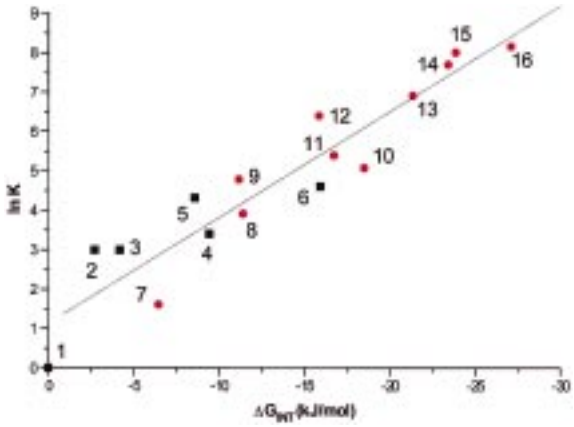

Figure 10. Relative sweetness ln K as a function of ΔG_{INT} (as calculated on the basis of model (b) for halogen derivatives of sucrose (squares) and 4-chloro-4-deoxy-*galacto*-sucrose (circles): $r = 0.94$ and $\sigma = 0.81$). The numbers correspond to Tables 3 and 4.

stant $\Delta G_{shift} = -6.8$ kJ/mol corresponding to the difference of the calculated logP values between sucrose and 4-chloro-4-deoxy-*galacto*-sucrose. Based on this argumentation the ΔG_{INT} values for the same series of sucrose and the 4-chloro-4-deoxy-*galacto*-sucrose halogen derivatives were calculated (Tables 3 and 4).

Plotting the natural logarithm of the sweetness data (ln K) versus the ΔG_{INT} values of the hydrophobic surface integral, we obtain a linear relationship with a regression line showing a correlation coefficient of $r = 0.94$ and $s = 0.81$ for the sucrose and the 4-chloro-4-deoxy-*galacto*-sucrose derivatives (Figure 10).

Our results clearly demonstrate that the MolFESD approach can be adequately used to localize hydrophobicity on the molecular surface. It allows a straightforward interpretation of individual contributions of local surface patches to the overall free energy value. It further renders a quantification of the hydrophobic contribution to the total free energy of association of different halogen substituted sucrose derivatives to a potential receptor, even if the receptor has not been identified yet.

Summary and conclusions

It has been demonstrated in this paper that the free energy of solvation of molecules in polarizable as well as apolar solvents can be represented as a surface integral over a suitably chosen function $\rho(S)$ – termed molecular free energy density, MolFESD – along the solvent accessible surface (SAS) of the molecule. The introduction of the MolFESD concept is justified by an electrostatic model describing the interaction of a molecular charge distribution with a continuous solvent separated by the SAS. This interaction can be transformed to a surface integral representation using the Gauss theorem. For nonpolar solutes the representation of the free energy of solvation is based on statistical arguments. This quantity becomes a surface extensive property if the ratio of solvent size to solute size tends towards zero. As a consequence of the result in the limiting cases we proposed that the MolFESD concept can generate reasonable estimates of solvation free energies or transfer free energies between two liquid solvents in all intermediate cases.

The MolFESD approach offers a localized picture of molecular hydrophobicity on the SAS with the benefit of a quantitative interpretation. This is accomplished by the fact that the MolFESD function can be composed of atom based contributions wherein membership functions control the individual surface dues. The parametrization has been established on the basis of numerous experimental data for the partition coefficient in a 1-octanol/water system.

The MolFESD concept has been applied to the calculation of the hydrophobic contribution ΔG_{INT} to the overall ΔG-value for the association of several halogen derivatives of sucrose and 4-chloro-4-deoxy-*galacto*-sucrose to the (still unknown) sweetness receptor. In our first approach the original receptor model of Shallenberger and Kier was modified by means of a highly flexible receptor pocket which is able to cover all of the hydrophobic part of the ligands. Taking a water-saturated 1-octanol solution as a hydrophobic biophase analogue, we have calculated ΔG_{INT}-values. The comparison between these values and the experimentally determined sweetness of the compounds exhibited a clear but non-linear correlation.

A second, refined model was formulated, wherein it was assumed that most of the fructose portion is covered by a flexible hydrophobic receptor pocket. In order to estimate the ΔG_{INT} values the integration over the MolFESD was done along the surface of the furanoid ring whereas other hydrophobic parts of the surface were taken as additive contributions. Having done the same comparison as stated above we found a linear correlation of experimental sweetness and ΔG_{INT}, proving the receptor model assumptions to be convenient. Therefore our model should be capable to predict relative sweetness for new compounds which only differ in modifications in the hydrophobic part of sucrose.

It is clear that the model approach presented here has its limitations if the microscopic structure of the solvation shell has to be taken into account and explicit calculations of conformational, energetic and entropic terms have to be performed. We are nevertheless optimistic that the MolFESD concept can be very efficiently used to generate new physical properties (like partial ΔG_{INT} values) which can be efficiently used in QSAR studies for the prediction of active compounds.

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