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A new approach to analysis and display of local lipophilicity/ hydrophilicity mapped on molecular surfaces

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SUMMARY

A new method for display and analysis of lipophilic/hydrophilic properties on molecular surfaces is presented. The present approach is based on the concept of Crippen and coworkers that the overall hydrophobicity of a molecule (measured as the logarithm of the partition coefficient in an octanol/water system) can be obtained as a superposition of single atom contributions. It is also based on the concept of molecular lipophilicity potentials (MLP) first introduced by Audry and coworkers in order to establish a 3D lipophilicity potential profile in the molecular environment. Instead of using a l/r- or an exponential distance law between the atomic coordinates and a point on the molecular surface, a new distance dependency is introduced for the calculation of an MLP-value on the solvent-accessible surface of the molecule. In the present formalism the Crippen values (introduced for atoms in their characteristic structural environment) are 'projected' onto the van der Waals surface of the molecule by a special weighting procedure. This guarantees that only those atomic fragments contribute significantly to the surface values that are in the close neighbourhood of the surface point. This procedure not only works for small molecules but also allows the characterization of the surfaces of biological macromolecules by means of local lipophilicity. Lipophilic and hydrophilic domains can be recognized by visual inspection of computer-generated images or by computational procedures using fuzzy logic strategies. Local hydrophobicities on different molecular surfaces can be quantitatively compared on the basis of the present approach.

1. INTRODUCTION

Attraction and repulsion between bioactive molecules are controlled by a variety of weak interactions: the van der Waals surfaces of the molecules should be complementary in the contact area, the local charge distributions should be of complementary sign close to the binding site in order to maximize Coulomb attraction, proton donor and acceptor should occur pairwise in close local relationship for building up intermolecular hydrogen bonds, and, finally hydrophobic parts of the molecules should be brought in close contact for an effective hydrophobic interaction [1].

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For the first three types of interactions reasonable model calculation can be performed – at least in principle. The hydrophobic interaction, however, cannot be treated within a reasonable model approach up to now, although it is generally accepted [1,2] that this type of interaction becomes essential in the case of large molecules like proteins in an aqueous environment. The importance of hydrophobicity in structural biology has been recognized for a long time [2] and has to be considered as an essential factor for drug–receptor interactions [1,3]. Hydrophobicity may be described as a combination of energetic and entropic effects. The first is related to differences in the energetics between a given molecule and the molecules in the solvation shells (of different solvents). The entropic effect is driven by the ordered orientation of solvent molecules at the solute–solvent interface if solute and solvent differ in their polarization (polarizability). This leads to an aggregation of non-polar (hydrophobic) compounds within aqueous (or – more generally –polar, i.e. hydrophilic) solvent, and vice versa. The association takes place in a way that the area of the hydrophobic–hydrophilic interface is kept at a minimal value.

The quasi-attractive interaction of organic compounds or non-polar groups in the water phase is known as the 'hydrophobic effect' [4]. It can be quantified using the Gibbs free energy change (ΔG) of transfer of an organic compound between an aqueous and an apolar phase.

$\Delta G_{transfer}$ can be considered as the key quantity to describe the hydrophobic effect, but this statement is not very helpful for an understanding of this effect within a model scenario since there is no simple way to estimate $\Delta G_{transfer}$. There is still no simple physical model available for hydrophobicity. However, there are several attempts to define relative lipophilicity values on the basis of empirical findings. Such values can be obtained for example from the partition coefficients (log P) of the sample in polar/apolar heterogeneous reference systems.

Fujita et al. [5] could prove, in a comprehensive study, the additive-constitutive nature of the partition coefficient. Based on these results hydrophobicity values can be established for any molecule as well as for arbitrary molecular fragments. The so defined lipophilicity of a molecule (measured, for example, by its log P value) can be regarded as the sum of increments related to the lipophilicities of its fragments, f_i:

$$\log P = \sum_i f_i \tag{1.1}$$

Nys and Rekker [6] and Rekker [7] gave some fragmental values for partition coefficients in the octanol/water system, followed by more detailed studies by Hansch and Leo [8]. Although other systems have also been used for hydrophobicity studies [9], the vast majority of measurements was carried out in the octanol/water system, leading to tables of lipophilicity values for a large number of molecular fragments.

One problem with the Hansch–Leo approach arises from the fact that correction factors for the f_i-values are required to cover the mutual influence of neighbouring fragments. Due to these correction factors, local contributions towards the partition coefficient are difficult to evaluate. Nevertheless this method was refined by Kellogg and Abraham [10] to calculate and predict hydrophobic interactions between atoms of different molecules. The alternative approach of Broto et al. [11] could avoid such correction factors by diminishing the fragments, but this method still does not include the explicit treatment of H-atoms. The last step towards an empirical quantification of lipophilicity contributions of individual atoms has been accomplished by Ghose and Crippen [12]. In their treatment the atomic contributions were defined by using a

classification of each atomic fragment (including hydrogen atoms) 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 [12–14] and this list is still being expanded.

2. THE MOLECULAR LIPOPHILICITY POTENTIAL

The methods described above are of great importance for the estimation of octanol/water partition coefficients of molecules which have not been studied experimentally, i.e. a molecule which has not been synthesized up to now can be checked with respect to an expected overall hydrophobicity. However, the formalisms do not give any suggestion towards a local measure of hydrophobicity. The manifestation of local hydrophobicity can be, nevertheless, of great importance for the generation of approximative models for the geometrical and energetical topology of a receptor site. This has recently been demonstrated by Lichtenthaler et al. [15], who studied the sweetness receptor. In this particular case it seems to be generally accepted that the receptor should contain a proton acceptor function, a proton donor function, and a hydrophobic functionality, all three arranged in a triangle ('the sweetness triangle'). While proton donor and acceptor functions can be easily identified in a molecular structure, this is not obviously possible for the hydrophobic interaction site. Questions like this require the localisation of a hydrophobic (or hydrophilic) interaction at least in a qualitative way. Such a localization is extremely helpful in computer-aided molecular design (CAMD) techniques. With modern computer-graphical tools, molecules can be visualized in a variety of different ways. Despite the traditional representations as chicken wire models, ball and stick models, or CPK models (intersecting spheres) a new standard has been established. This is the representation of the molecular surface as it is seen from a test particle [16]. Examples are the solvent-accessible surface or the contact surface of Connolly [17]. Such surfaces represent the repulsive interaction between molecules. Moreover, they can be used as screens in order to map local molecular properties (like the electrostatic potential of a unit charge) by a colour-coded representation. Such a graphical representation of molecular properties on the van der Waals surface or the solvent-accessible surface became an important tool in molecular modelling [18,19]. The subdivision of molecular lipophilicity into fragmental contributions is a prerequisite for the manifestation of local hydrophobicity, and a surface map of this quantity. It seems to be reasonable to postulate a distance-dependent rule for the influence of different fragments on the lipophilicity at a certain surface point. One possible function for this rule has been proposed by Audry et al. [20]. They introduced the name 'molecular lipophilicity potential' (MLP), and postulated (in analogy to the electrostatic potential function) a functional form

$$\mathrm{MLP} = \sum_i f_i / (1 + d_i) \tag{2.1}$$

where f_i is the partial lipophilicity of the i-th fragment of a molecule and d_i is the distance of the measured point in 3D space from the center of fragment i. The long-range distance dependence of this function is that of a Coulomb potential, i.e. a large number of atoms contributing to an MLP value at a certain point of the molecular surface may be dominantly determined by a large number of contributions which are far away from this point. Despite the lack of any physical

basis for this type of distance dependency of the MLP, useful results could be obtained from the application of this function for visualization of molecular lipophilicity [21,22] for sufficiently small molecules (where the number of contributions is small). Furet et al. [21] were able to display MLP profiles on the van der Waals surface of the 20 proteinogenic amino acids using Crippen's single atom fragment lipophilicities. Their results are in agreement with the concept of a 'hydrophobic dipole moment' introduced by Eisenberg et al. [23] and Eisenberg and McLachlan [24].

Fauchère et al. [25] proposed another form for the MLP. They defined an exponential distance dependence for fragmental contribution: MLP ~ exp(−d). This potential function was designed on the basis of the proximity effects observed by Nys and Rekker [6], Rekker [7] and Hansch and Leo [8] and was used for the examination of a drug–receptor system.

Both methods mentioned above have been successfully applied to small molecular systems. Brasseur could extend the approach to helical peptides, calculating isopotential contours of hydrophobicity [26] by projecting the atomic partial values on the van der Waals surface, starting from there with an exponential decrease to any point in space.

Despite the success of the MLP approach for small molecules, there is no physical reason for the use of one or another distance dependency in this 'potential' at the same time as using the partial log P values of Crippen and coworkers as 'partial charges' located at the positions of the atoms in a molecule. As mentioned above, a long-range distance dependency of the individual potential contributions may lead to overcompensation of local effects (see Fig. 1).

Since the incrementation of the log P values is based on local contributions, overcompensation of these local effects would be in contradiction with the method for determining f_i values by statistical procedures.

In this paper we propose an MLP approach which was particularly designed for mapping local hydrophobicity values on a solvent-accessible surface of a molecule based on the atomic increment values of Crippen and others [12–14]. Like the above-mentioned authors, we also introduced the MLP as a superposition

$$MLP_s = N^{-1} \sum_i f_i \, g(d_i) \tag{2.2}$$

with some function g(d) which is dependent on the distance d of a surface point from the origin of an atomic increment i of the molecule, and a 'normalization' factor

$$N = \sum_i g(d_i) \tag{2.3}$$

The function g(d) has no physical meaning. It should only fulfil two conditions: (i) It should be smooth and have finite values for $d < d_{cut\text{-}off}$ where $d_{cut\text{-}off}$ is some cut-off value which is termed the *proximity distance*. The value of $d_{cut\text{-}off}$ should be larger than any van der Waals radius of an atom in the molecule under consideration. (ii) For distances $d > d_{cut\text{-}off}$ the function values of g(d) should rapidly tend towards zero.

As mentioned above, the MLP_s function in Eq. 2.2 is not based on a rigorous physical concept. However, for the visualization of lipophilicity values on a molecular surface it is only necessary that the function generates reasonable data for all surface points. This is guaranteed in the present case by the normalization function N. The function MLP_s represents a weighted average of all the f_i values of atomic increments i for which $d_i < d_{cut\text{-}off}$ is fulfilled. All atoms which are further away

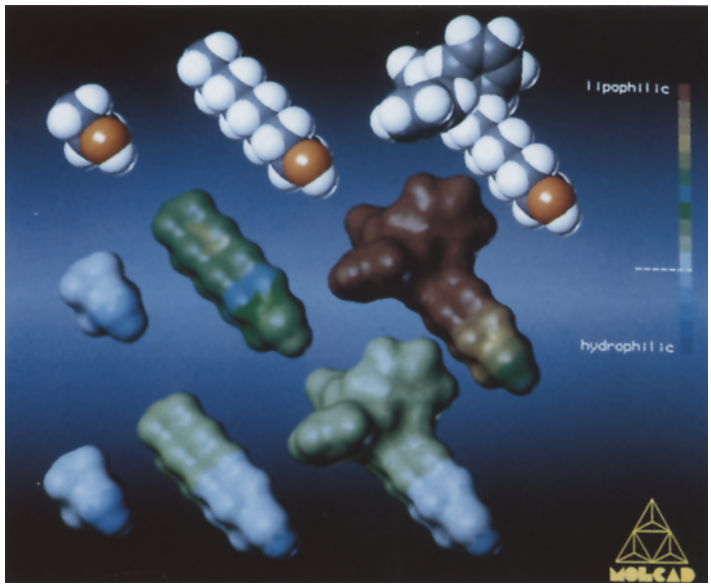

Fig. 1. Influence of the distance of molecular fragments on the local MLP values on the molecular surface. Ethanol, octanol, and triphenyloctanol (TPO) (from left to right) are displayed as CPK and solvent-accessible contact surface models, using MLP definitions given in Eq. 2.1 (MLP_c, second row) and Eq. 2.2 (MLP_s, third row). The colour coding is ranged between the minimum and the maximum value of the surface potential in the two different calculation series (in total). It varies from hydrophilic (blue) to lipophilic (brown). For the small compound ethanol, the MLP images calculated with respect to Eq. 2.1 and Eq. 2.2, respectively, look very similar, but they become increasingly different when hydrophobic fragments are added. With increasing size of the molecule, the local MLP_c values (Eq. 2.1) become very large as a consequence of the summation of many fragmental contributions which do not decay fast enough with increasing distance from the incremental centre. Applying this function, the hydrophilicity of the OH group is neutralized in octanol, and this group becomes even hydrophobic by the long-distance influence of the lipophilic phenyl substituents of TPO. With the MLP_s representation local hydrophilicity is conserved. Nearby hydrophobic CH_2 groups reduce the hydrophilic character of the OH group, but substituents at long distance do not have a measurable influence.

from the surface point do not contribute significantly. This can be demonstrated by a few simple examples (see Fig. 2).

(a) If only one atomic centre **1** is located within a proximity distance $d_{cut\text{-}off}$ (see Fig. 2a) from a surface point **S**, only this atom contributes significantly to the MLP_s at this point, i.e.

$$\sum_i f_i\, g(d_i) \cong f_1\, g(d_1) \tag{2.4a}$$

and

$$N = \sum_i g(d_i) \cong g(d_1) \tag{2.4b}$$

and consequently

$$MLP_s = f_1. \tag{2.5}$$

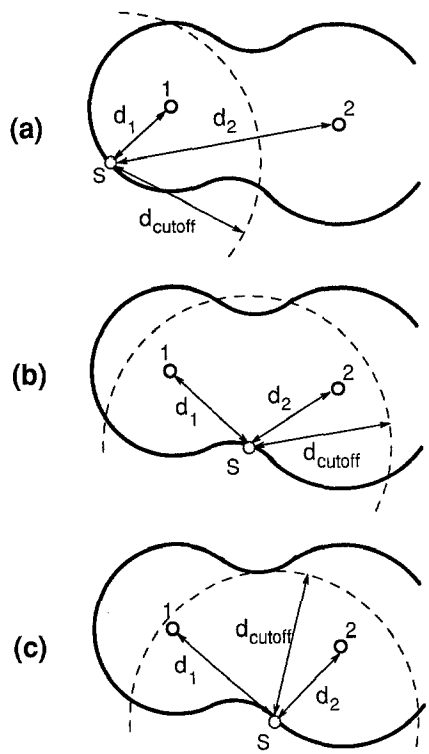

Fig. 2. Fragmental influence on the local MLP_s values (schematically). (a) The incremental value is mapped onto the surface if there is only one centre within a distance $d_i < d_{cut\text{-}off}$ from the surface point. (b) The arithmetic mean of two incremental values is mapped onto the surface if there are two centres of equal distance $d_i < d_{cut\text{-}off}$ from the surface point. (c) A weighted average of two incremental values is mapped onto the surface if there are two centres of different distance $d_i < d_{cut\text{-}off}$ from the surface point.

The increment value f_1 is mapped on the surface point **S**.

(b) If two atomic centres **1** and **2** are located within the distance $d_{cut\text{-}off}$ and a surface point is chosen in such a way that $d_1 = d_2$ (see Fig. 2b), only these two will contribute significantly to the MLP_s at **S**, i.e.

$$\sum_i f_i\, g(d_i) \cong f_1\, g(d_1) + f_2\, g(d_2) = (f_1 + f_2)\, g(d_1) \tag{2.6a}$$

and

$$N = \sum_i g(d_i) \cong g(d_1) + g(d_2) = 2g(d_1) \tag{2.6b}$$

and consequently

$$MLP_s = (f_1 + f_2)/2. \tag{2.7}$$

The MLP_s value becomes equal to the arithmetic mean of the two increment values f_1 and f_2.

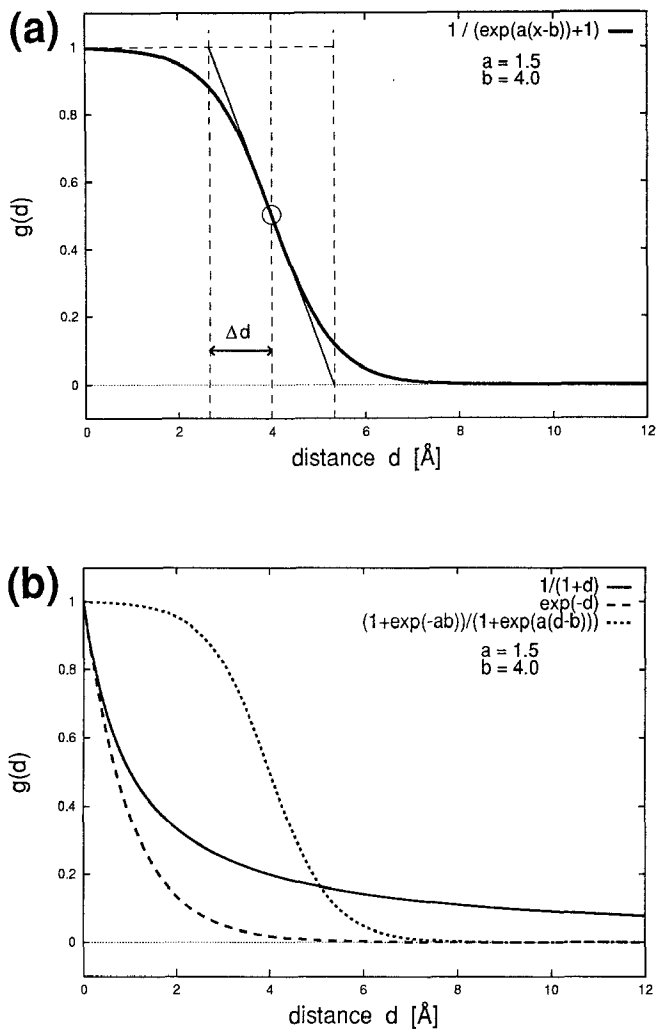

Fig. 3. (a) Fermi type distance function g(d). The function decays from values $d \approx 1$ to $d \approx 0$ within a distance interval of $2\Delta d$. $\Delta d = 2/a \approx 1.33$ Å. (b) Three different distance functions g(d) are used for MLP definitions.

(c) For the case where, as in Fig. 2b, only two contributions are relevant but the distances d_1 and d_2 are different (see Fig. 2c), one obtains analogously

$$MLP_s = \frac{f_1 g(d_1) + f_2 g(d_2)}{g(d_1) + g(d_2)} \tag{2.8}$$

i.e. the MLP_s values are a weighted average of f_1 and f_2 with the weighting factors

$$p_1 = \frac{g(d_1)}{g(d_1) + g(d_2)} \tag{2.9a}$$

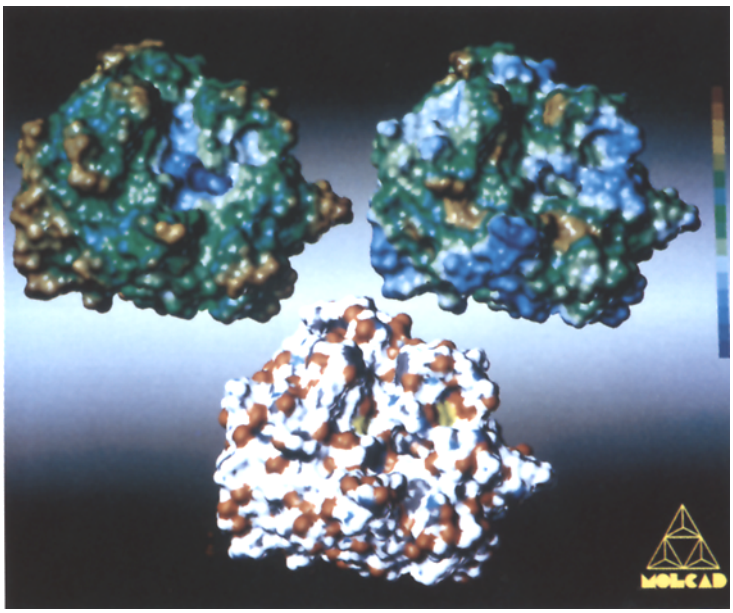

Fig. 4. Trypsin with different MLP representations. Solvent-accessible contact surface models of the digestive enzyme trypsin with MLP_c potential (top left), MLP_s potential (top right), and coloured by atom contributions (bottom). Each MLP representation is scaled over the complete colour range from minimum (blue) to maximum lipophilicity (brown). The orientation of the molecule is chosen such that the specificity pocket responsible for enzyme–substrate (or inhibitor) recognition is located on the front side. It can be seen easily that in the MLP_c representation the influence of lipophilicity partial values is overcompensated by topological effects, considering topologically exposed surface regions (i.e. separated from the overall hydrophilic bulk) as relatively hydrophobic, while clefts and pockets are regarded as hydrophilic (i.e. embedded in the protein bulk). MLP_s on the other hand represents a picture which is in accordance with chemical intuition.

$$p_2 = \frac{g(d_2)}{g(d_1) + g(d_2)} \tag{2.9b}$$

which now depend on the explicit form of g(d).

The examples demonstrate that the MLP_s approach generates weighted averages of the f_i data of Crippen and others. The quantities

$$p_i = \frac{g(d_i)}{\sum_j g(d_j)} \tag{2.10}$$

can be interpreted as probablities

$$\sum_i p_i = 1$$

or weighting factors for the contributions of individual atomic or molecular increments to the local MLP_s value

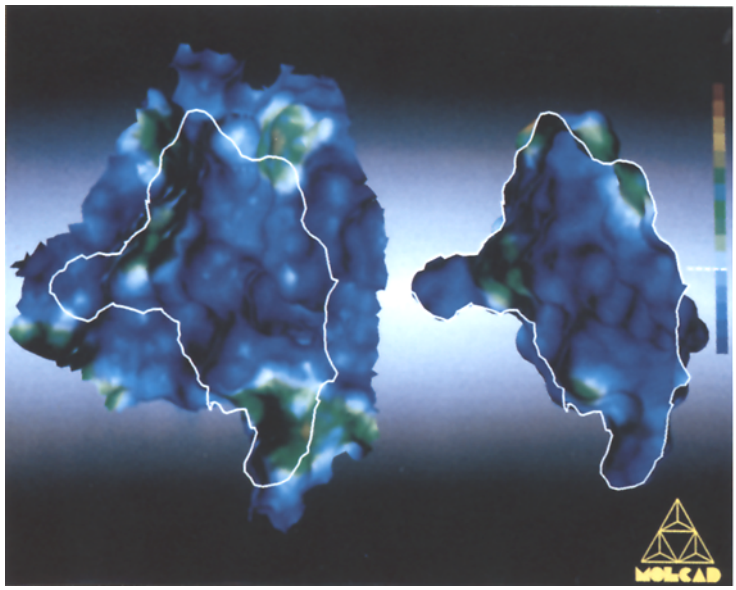

Fig. 5. Contact region of the trypsin–PTI complex showing MLP_s lipophilicity patterns. The trypsin surface is seen from outside, the PTI surface from inside the molecule. The PTI surface contour is projected as a white line upon the corresponding trypsin surface region (data from bovine pancreatic β-trypsin–BPTI complex X-ray structure, Brookhaven file 2PTC).

$$MLP_s = \sum_i p_i f_i . \tag{2.11}$$

There are several possibilities to choose the distance function g(d). This work is focused on a visual representation of local hydrophobicity. We have considered a function which is constant for $d < d_{cut\text{-}off} - \Delta d$ and then decays within $d_{cut\text{-}off} - \Delta d < d < d_{cut\text{-}off} + \Delta d$ towards zero wherein Δd is a conveniently chosen distance value (see Fig. 3). In the next section an analytical representation of such a function is presented and some results are discussed.

3. REPRESENTATION OF THE MLP_s FUNCTION

As there is no physical reason for a certain distance law for the function g(d), we have chosen an analytical one in the form of a Fermi function [27] which guarantees that the two conditions (i) and (ii) are fulfilled:

$$g(d) = \frac{1}{\exp[a(d - d_{cut\text{-}off})] + 1} \tag{3.1}$$

which decay from values close to unity to values close to zero in the interval

$$d_{cut\text{-}off} - \Delta d < d < d_{cut\text{-}off} + \Delta d \tag{3.2}$$

with

$$\Delta d = 2/a \tag{3.3}$$

(see fig. 3a). In this representation the distance function is completely determined by the proximity distance $d_{cut\text{-}off}$ and the range $2\Delta d$ wherein the function decays.

The consequences of this particular choice for g(d) are obvious. This can again be demonstrated with the aid of a simple example. Let us assume as in case (c) from Section 2 that only two atomic increments are contributing to the MLP_s at a surface point S and that both distances d_1 and d_2 obey the inequality expression

$$d_i < d_{cut\text{-}off} - \Delta d \tag{3.4}$$

then $g(d_1) \approx g(d_2)$ results from the functional form of g and one has with Eqs. 2.8 and 2.9

$$MLP_s(S) \cong (f_1 + f_2)/2 \tag{3.5}$$

as in the general case (b).

For full contribution of a fragmental value f_i (i.e. g(d) = 1) in the case of d = 0 we introduced a correction factor to the Fermi function:

$$g(d) = \frac{\exp[-a \cdot d_{cut\text{-}off}] + 1}{\exp[a(d - d_{cut\text{-}off})] + 1} \tag{3.6}$$

It has been mentioned that the MLP_s has no physical meaning per se. However, the general approach is very flexible, i.e. with a reasonable choice of the free parameters physical properties can be considered. We think that our approach is a generalization of previous works. Taking for example the proximity distance roughly equal to typical van der Waals radii, our approach generates similar MLPs as those proposed by Brasseur [26]. This author postulated a function which has the f_i value on the surface of a CPK type model and decays exponentially with increasing distance.

4. APPLICATIONS

The model approach presented above has been applied to a variety of molecules from small organic compounds (see, for example, Ref. 15) to large biomolecules.

For small molecules the visualization of the MLP on a solvent-accessible surface looks quite similar whatever MLP is used (see Fig. 1). However, for biomolecules like proteins considerable differences occur. This is demonstrated in Figs. 4 and 5, where the surfaces of PTI (pancreatic trypsin inhibitor) and trypsin are shown with colour-coded MLP values calculated with respect to the old formula Eq. 2.1 and with g-functions as described in Section 3. In the latter we used a proximity distance of $d_{cut\text{-}off}$ = 4 Å and Δd = 2 Å. The cut-off value takes into account that in the original parametrization of the f_i values the next and second next neighbours have been considered for the definition of the atom type [12–14].

It is seen that there are large hydrophobic and hydrophilic domains on the surface of the

TABLE 1
COMPUTATION TIMES FOR MLP CALCULATION FOR PROTEIN SURFACES ON AN IRIS Indigo R4000 WORKSTATION

Molecule	No. of atoms	No. of surface dots	CPU time (s)
Lysozyme	1961	15872	184
Elastase	3590	25890	550
HyHEL-10 antibody (F_{ab})	6449	47162	1835

proteins considered here. These domains cannot only be identified by visual inspection but also by systematic algorithms using fuzzy logic strategies. This is demonstrated in a separate paper [28] in a more general context.

5. CONCLUSIONS AND OUTLOOK

Although hydrophobic (or hydrophilic) interactions between molecules or molecular fragments can in principle be described on the basis of ΔG calculations, there is no model approach available which allows this form of weak interaction to be treated in a consequent manner. However, it has been demonstrated [15,21,22,25,26] that the hydrophobicity of a molecule can be sufficiently described (at least in a qualitative manner) within empirical concepts based on partition coefficients measured in an octanol/water system. We have demonstrated in the present paper that a Fermi type distance function in so-called lipophilicity potentials (MLP) can be used to map weighted increment values f_i for Crippen's log P data onto the molecular surfaces in a straightforward way. The procedure is particularly useful for the identification and comparison of local hydrophilic and hydrophobic areas of large biomolecules and smaller substrates for which long-range distance functions [20,21] definitely fail. Up to now there is no quantitative interpretation of the new MLP_s function, but there is a very promising approach towards it. The surface integral over the MLP_s agrees within an error of 20% with the overall log P value as calculated with Crippens method [12]. We are currently working on a reparametrization of atomic increment values f_i where now the fitted function Eq. 1.1 is replaced by a surface integral [29]. With f_i values determined in the latter procedure one should expect to calculate the relative hydrophobicities of different conformations of one molecule, and so have a tool in hand to study the influence of the solvation on the molecular structure within a model scenario which is easy to handle.

Typical computation times for protein surfaces of different size are shown in Table 1.

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