

Dry and wet interfaces: Influence of solvent particles on molecular recognition

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We present a coarse-grained lattice model to study the influence of water on the recognition process of two rigid proteins. The basic model is formulated in terms of the hydrophobic effect. We then investigate several modifications of our basic model showing that the selectivity of the recognition process can be enhanced by considering the explicit influence of single solvent particles. When the number of cavities at the interface of a protein-protein complex is fixed as an intrinsic geometric constraint, there typically exists a characteristic fraction that should be filled with water molecules such that the selectivity exhibits a maximum. In addition, the optimum fraction depends on the hydrophobicity of the interface so that one has to distinguish between dry and wet interfaces.

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I. INTRODUCTION

Molecular recognition denotes the ability of a certain biomolecule to find the right partner molecule in a heterogeneous environment, such that the formed complex can perform its assigned biological task. Prominent examples of specific recognition processes between proteins comprise enzyme-substrate binding, antigen-antibody binding, or protein-receptor interactions [1,2]. It is a remarkable property of recognition processes that a biomolecule (called probe molecule throughout this paper) can identify its “correct” complex partner by distinguishing between the supposed “target” and a competing “rival” molecule that possibly features only a slightly different structure at the binding epitope. Therefore, an understanding of molecular recognition processes is obviously not only interesting from a biological point of view, but also necessary for various biotechnological or pharmaceutical applications. The high specificity of molecular recognition processes can be illustrated by the “lock-and-key” mechanism for inflexible biomolecules which demands a high geometrical complementarity for the two molecules forming a complex [3,4]. For that reason, there is in general only one possible binding partner (say “key”) for a given molecule (“lock”). As most macromolecules prove to be flexible, the so-called “induced-fit” scheme has been established, according to which the necessary complementarity is only achieved after some conformational changes in the corresponding backbones of the proteins [5].

The forces that stabilize a protein complex basically emerge from a complicated interplay between noncovalent bonds. These bonds are characterized by energies of the order of 2–6 kcal/mol [6]. Since this is only slightly stronger than the thermal energy $k_B T_{\text{room}} \approx 0.62$ kcal/mol at physiological conditions, we can conclude that the formation of a stable protein complex demands a large number of noncovalent bonds and thus many participating functional groups with appropriate complementarity [4]. It has been investigated that the driving forces for molecular recognition are dominated by hydrogen bonds and especially by the hydrophobic effect [2,7–10]. The hydrophobic effect sums up the mechanism that the hydrophobic residues of proteins are ef-

fectively pushed together when the polar solvent leaves the space between the hydrophobic amino acids for entropical and energetical reasons [11].

The enormous significance of water for biological systems has been manifest for many years [12]. Although water is essential for the structure, stability, dynamics and functions of biomolecules, biological models often describe the solvent only as a passive component of the system as is done, for example, by referring to the hydrophobic effect. However, it has been shown that water molecules which are embedded in cavities between two bounded proteins play a crucial role for the formation and stabilization of the complex and can thus be considered as an active part of the structure [9,12–17]. Indeed it has been observed that in interfaces between two proteins about 10%–20% of the area is made up of cavities on average of which a large number are filled by at least one water molecule [8,18,19]. The energetic contributions of the buried water molecules are basically twofold. They can either contribute van der Waals interactions with adjacent amino acids or form hydrogen bonds between constituents of the two proteins (sometimes involving more than one buried water molecule). The latter possibility requires a high degree of geometric directionality of the involved molecules and parts of the proteins. The energetic contributions due to these mediated interactions are typically smaller by a factor of 2 or 3 than direct contacts; however, examples where they are of the same strength as direct contacts do exist [13,15,17].

Interfaces of protein complexes show different levels of hydration and can exhibit up to as many interactions caused by embedded water molecules as by direct hydrogen or salt bridges [16]. On experimental grounds one can basically distinguish between “wet” interfaces with many embedded water molecules and “dry” interfaces where water is absent [9,16,20]. Dry interfaces typically feature a ring of water molecules around the binding epitope. In general the less hydrophobic interfaces between antibodies and antigens tend to be wet whereas the more hydrophobic protease-inhibitor interfaces appear to be dry. This suggests a correlation between the hydrophobicity of the interface and the degree of hydration. Note, however, that exceptions to this broad rule do exist.

In this paper we will investigate the influence of buried water molecules in protein-protein interfaces on the selectivity of the corresponding recognition process. Our considerations are carried out within a coarse-grained approach where the bulk solvent degrees of freedom are integrated out. The energetics is then formulated on the level of amino acids in terms of the hydrophobic effect between residues of different hydrophobicity. Additional residual water degrees of freedom which are embedded in the interface and can thus actively mediate interactions between amino acids are then incorporated into the model. From the point of view of modeling this can be done by applying direct and water-mediated contact energies [15] or by using generic double well potentials of mean forces with one minimum corresponding to direct contacts of two residues and a characteristic second one resulting from water-separated contacts [12,14]. We finally remark that the problem of molecular recognition has been considered in coarse-grained approaches in several articles [21–30].

For our investigations we utilize a general two-stage approach (Sec. II) where in a first step an ensemble of probe molecules is designed with respect to a given target. In a second step, we investigate the recognition ability or selectivity of the probe ensemble by comparing the associated free energy for the two cases that the probe molecules bind the target or a different rival molecule, respectively. In the subsequent sections, we will modify the “elementary” hydrophobic-polar (HP) model by taking the direct influence of single solvent molecules into account. Nevertheless, we have to keep in mind that the protein interaction with water is already part of the HP model since its energetics is based on the hydrophobic effect. In the following sections we analyze the influence of buried water molecules in the interface on the selectivity of molecular recognition. Whereas in Sec. III every cavity at the interface is filled by a water molecule, in Sec. IV we make the inclusion optional and additionally couple the water’s interaction to the adjacent type of amino acid. In particular, we will investigate whether or not the inclusion of solvent molecules in the interface can lead to an enhancement of the selectivity. The technical details of how the selectivity for the model with an optional inclusion of water is calculated are discussed in the Appendix.

II. GENERAL APPROACH TO MOLECULAR RECOGNITION

In this section we briefly discuss how we model the recognition process and introduce a measure of its selectivity (more detailed accounts can be found elsewhere [26,29,30]). We model a protein’s recognition site at the interface of a protein-protein complex as a two-dimensional array of N amino acids, also called residues or monomers. Typical values of N range between 30 and 60 [2]. For the description of a so called probe molecule θ , which is supposed to recognize a certain target molecule, we introduce the N -dimensional vector $\theta=(\theta_1, \dots, \theta_N)$, whose i th component indicates the type of amino acid on site i . Accordingly, the target molecule σ is specified by its residues $\sigma=(\sigma_1, \dots, \sigma_N)$. For the sake of simplicity we assume that both proteins have the same num-

ber of monomers at the interface which match when forming a complex. We note, however, that systems where this assumption holds true do exist [31].

To specify a single residue one should *a priori* distinguish between the 20 different amino acids occurring in nature. In the coarse-grained approach of the HP model, we reduce the alphabet of amino acids to only two letters and differentiate between the polar and nonpolar (hydrophobic) subgroup. Thus we get an Ising-type variable and choose the convention to attribute to σ_i or correspondingly θ_i the value $+1$ for a hydrophobic (H) and -1 for a polar (P) monomer at site i . We justify this procedure by having in mind that hydrophobicity acts as the dominant driving force in molecular recognition [2,9,10]. Furthermore one gets the two amino acid subgroups as a very good approximation by applying an eigenvalue decomposition of the Miyazawa-Jernigan matrix which consists of the pairwise interactions between all natural amino acids [32,33]. Note that there exist also other methods to reduce the alphabet of amino acids to five clustered subgroups [34,35].

The induced-fit theory motivates us to account for minor rearrangements of amino acid side chains which provide the needed complementarity for the formation of a protein-protein complex. This feature is incorporated into the model by defining the quality of contact between the binding partners labeled as $S=(S_1, \dots, S_N)$. We just discriminate between “good” ($S_i=+1$) and “bad” ($S_i=-1$) contacts at site $i=1, \dots, N$. The (geometric) quality of the contact can be understood as a characteristic trait of one of the molecules or, alternatively, as a collective variable of the probe and target molecule. The contact variable sums up all geometric conditions at the interface, for example, the distances between opposite residues or the alignment of their polar moments. Its relevance for the inclusion of water molecules at the interface is discussed in Secs. III and IV.

In our picture of the protein complex, we consider a general Hamiltonian $\mathcal{H}(\sigma, \theta; S)$ depending on the structures σ and θ and some kind of interaction between binding partners at position i which is related to the corresponding variable S_i . We formulate the energetics at the interface by a modified HP model [26]:

$$\mathcal{H}(\sigma, \theta; S) := -\varepsilon \sum_{i=1}^N \frac{1+S_i}{2} \sigma_i \theta_i. \quad (1)$$

The parameter $\varepsilon > 0$ gives the strength of the hydrophobic interaction and is typically of the order of 2 kcal/mol [31]. Note that the factor $\frac{1+S_i}{2} \in \{0, 1\}$ suppresses the contribution of binding energy in the case of bad contacts. For a good contact at site i we receive the contribution $-\varepsilon \sigma_i \theta_i$; if the type of residues of the protein interface in contact is identical, i.e., $\sigma_i \theta_i = 1$, we will get a favorable term $-\varepsilon < 0$, whereas for different types of amino acids the resulting $+\varepsilon$ represents a nonfavorable energy contribution. We note that HP-like models have been applied in various biophysical contexts over the last years [36–44].

To study the recognition process between the two biomolecules, we adopt a two-stage approach. In the first step, also referred to as the design step, we prepare an ensemble of

probe molecules θ which are supposed to recognize a given and fixed target $\sigma^{(T)} = (\sigma_1^{(T)}, \dots, \sigma_N^{(T)})$. For every possible configuration θ of the probe molecule we therefore assign a conditional probability of its occurrence $P_D(\theta|\sigma^{(T)})$. We describe the conditions of the system by the Lagrange multiplier $\beta_D \geq 0$ and demand a canonical Boltzmann distribution

$$P_D(\theta|\sigma^{(T)}) = \frac{1}{Z_D} \sum_{\{S\}} \exp[-\beta_D \mathcal{H}(\sigma^{(T)}, \theta; S)], \quad (2)$$

where the partition function Z_D guarantees the normalization $\sum_{\{\theta\}} P_D(\theta|\sigma^{(T)}) = 1$. The sum in Eq. (2) extends over all 2^N possible configurations of S . This design step has been introduced to mimic the process of evolution in nature or design in biotechnological applications. We remark that the parameter β_D , which can be interpreted formally as an inverse temperature in our simplifying view of evolution or biotechnological design, basically controls the degree of optimization of the probe with respect to the target [29]. As recognizing biomolecules are usually well optimized to each other we typically choose a fairly large value for β_D .

In the second step of our approach, we test the recognition ability of the designed ensemble. To this end, we consider two copies of the ensemble of probe molecules at the inverse temperature $\beta \geq 0$: one ensemble is given the target molecule $\sigma^{(T)}$, the other system interacts with a competitive rival molecule $\sigma^{(R)} = (\sigma_1^{(R)}, \dots, \sigma_N^{(R)})$. At that point, we simulate that the probe molecules have to find their right partner and must decide between the formation of a complex with the target or the rival. Our aim is to calculate the free energies of the two possible protein complexes and the lower one is then realized in nature. First we evaluate the free energy for the complex consisting of target or rival and a fixed probe molecule θ :

$$F(\theta|\sigma^{(\alpha)}) = -\frac{1}{\beta} \ln \sum_{\{S\}} \exp[-\beta \mathcal{H}(\sigma^{(\alpha)}, \theta; S)], \quad (3)$$

for $\alpha \in \{T \equiv \text{target}, R \equiv \text{rival}\}$. Afterward we average over the ensemble of probe molecules using the conditional probability from the design step and get

$$F^{(\alpha)} = \sum_{\{\theta\}} F(\theta|\sigma^{(\alpha)}) P_D(\theta|\sigma^{(T)}). \quad (4)$$

For further investigations we consider the difference of the free energy $\Delta F(\sigma^{(T)}, \sigma^{(R)}) = F^{(T)} - F^{(R)}$ as a measure for the selectivity of the recognition process. For $\Delta F(\sigma^{(T)}, \sigma^{(R)}) < 0 \Leftrightarrow F^{(T)} < F^{(R)}$ the target is recognized by the probe molecules.

Since we have decided to describe molecular recognition on a very coarse-grained level it is quite natural that we will also average the difference in the free energy $\Delta F(\sigma^{(T)}, \sigma^{(R)})$ over all possible structures of target and rival molecules. Assuming a uniform probability distribution for both the target's and the rival's structure, one receives a result $\langle \Delta F \rangle$ that does not depend on specific configurations anymore. This number can be interpreted as a characteristic selectivity of the model and its associated Hamiltonian.

Let us end this section with a brief comment on the restriction of the contact variable S_i to two distinct values. At first glance, it might seem that the distinction between only

good and bad contacts is too simple and naive. So one could suggest to consider a finite number of discrete levels that interpolate between the extreme case of a good and a bad contact. This modification accounts for the fact that there is usually a considerable number of possible alignments between the corresponding polar moments of opposite amino acids, for example. It turns out that the selectivity depends in general on the structural information contained in the variables $\sigma^{(T)}$ and $\sigma^{(R)}$. However, different models of the contact variable do not change the corresponding functional dependence, although coefficients might be altered. Thus qualitative conclusions about the behavior of the selectivity remain the same. Therefore the simplifying reduction to two different states of the quality of a contact suffices to describe molecular recognition in the context of the presented approach. One can show that the result of $\Delta F(\sigma^{(T)}, \sigma^{(R)})$ is even the same for nonuniformly distributed (discrete or continuous) contact variables, as long as the distribution is symmetric with respect to the value lying in the middle between the values for good and bad contacts [45].

III. UNSPECIFIC INCLUSION OF INTERFACE WATER

In this paper we are mainly concerned with the effect of *embedded* solvent molecules at the interface of protein-protein complexes on the selectivity of molecular recognition. Our basic approach is based on the hydrophobic effect where bulk solvent degrees of freedom are already integrated out. The residual solvent degrees of freedom that show up at the interface as an active part have to be modeled explicitly. In our approach a solvent molecule can be embedded at a position where a bad contact appears. Hence the contact variable S_i describes the appearance of cavities at the interface. In this section we will relate the emergence of cavities to thermal fluctuations. In Sec. IV cavities will be modeled as an intrinsic geometric feature that is not liable to thermal fluctuations so that their number is fixed.

Let us allow for an existing cavity to be always filled by a water molecule that interacts somehow unspecifically with the adjacent amino acids so that the energy contribution does not distinguish between the types of the amino acids. This might be interpreted as a van der Waals contribution which has to be distinguished from a hydrogen bond that requires certain geometrical and structural prerequisites. To account for the geometrical conditions we consider favorable ($-\gamma < 0$) or unfavorable ($\gamma > 0$) energy contributions and thus introduce the variable $w = (w_1, \dots, w_N)$ with $w_i \in \{-1, 1\}$ for the solvent degree of freedom to distinguish between a favorable ($w_i = 1$) and an unfavorable ($w_i = -1$) energy contribution. The Hamiltonian then consists of a sum due to the direct contacts at the proteins' interface as modeled in Eq. (1) and a second term due to the burial of water molecules at bad contact sites:

$$\mathcal{H}(\sigma, \theta; S, w) := -\varepsilon \sum_{i=1}^N \frac{1+S_i}{2} \sigma_i \theta_i - \gamma \sum_{i=1}^N \frac{1-S_i}{2} w_i. \quad (5)$$

Consistent with observations (e.g., [12,13,17]) we request the ratio ε/γ to be typically of the order of 2–3. For a good

contact at site j so that water molecules cannot be embedded, the variable w_j corresponds to a water molecule of the bulk and delivers an entropic contribution as it appears in the summation for the partition function but provides no energy contribution.

We start with the design of the probe ensemble. The calculation of the conditional probability $P_D(\theta|\sigma^{(T)}) = \frac{1}{Z_D} \sum_{\{S\}} \sum_{\{w\}} \exp\{-\beta_D \mathcal{H}(\sigma^{(T)}, \theta; S, w)\}$ gives

$$P_D(\theta|\sigma^{(T)}) = \frac{\prod_{i=1}^N \{\exp[\beta_D \varepsilon \sigma_i^{(T)} \theta_i] + \cosh(\beta_D \gamma)\}}{\left\{ 4 \cosh\left[\frac{\beta_D}{2}(\varepsilon + \gamma)\right] \cosh\left[\frac{\beta_D}{2}(\varepsilon - \gamma)\right] \right\}^N}. \quad (6)$$

Before giving the result for the difference in the free energy we want to have a look at some observables of the system which characterize the design step. We define the complementarity K of the target $\sigma^{(T)}$ and a certain probe molecule θ as $K = \sum_{i=1}^N \sigma_i^{(T)} \theta_i$, whose possible values range from $-N$ to N . A value of K close to the maximum N means a high structural complementarity so that we expect the formation of a complex between target and probe to become energetically favorable. We can convert probability (6) into a distribution for the complementarity according to $P_D(K) = \sum_{\{\theta\}} P_D(\theta|\sigma^{(T)}) \delta_{K, \sum_{i=1}^N \sigma_i^{(T)} \theta_i}$. Using that result to calculate an averaged complementarity of the designed structures θ (for fixed target $\sigma^{(T)}$) according to $\langle K \rangle = \sum_{K=-N}^N K P_D(K)$, we finally arrive at

$$\langle K \rangle = N \frac{\sinh\left(\frac{\beta_D \varepsilon}{2}\right) \cosh\left(\frac{\beta_D \varepsilon}{2}\right)}{\cosh\left[\frac{\beta_D}{2}(\varepsilon + \gamma)\right] \cosh\left[\frac{\beta_D}{2}(\varepsilon - \gamma)\right]}. \quad (7)$$

Note in particular that the resulting expression for $\langle K \rangle$ is independent of the given target $\sigma^{(T)}$. Equation (7) provides an interpretation for the design parameter β_D , since for large $\beta_D \rightarrow \infty$ one gets $\langle K \rangle \rightarrow N$, i.e., the probe molecules are well optimized with respect to the fixed target and we thus talk of optimal design conditions. Further information that we can extract from Eq. (7) concerns the influence of the interaction between the proteins and the water given by the parameter γ . As the complementarity is decreased for increasing $\gamma > 0$, we can already expect the selectivity of the recognition to decay as well (compare Fig. 1).

Another observable of interest is the number $L = \frac{1}{2}(N - \sum_{i=1}^N S_i)$ of cavities at the interface. Instead of L we consider the normalized quantity

$$l_{\sigma^{(T)}} = \frac{1}{2} \left(1 - \frac{1}{N} \sum_{\{\theta\}} P_D(\theta|\sigma^{(T)}) \left\langle \sum_{i=1}^N S_i \right\rangle_{\sigma^{(T)}, \theta} \right) \quad (8)$$

for a certain target $\sigma^{(T)}$. The pointed angles $\langle \cdot \rangle$ in Eq. (8) denote a thermal average with respect to the fluctuating variables S and w ; the indices indicate that the structures $\sigma^{(T)}$ and θ are kept fixed. The result for $l_{\sigma^{(T)}}$ proves to be independent

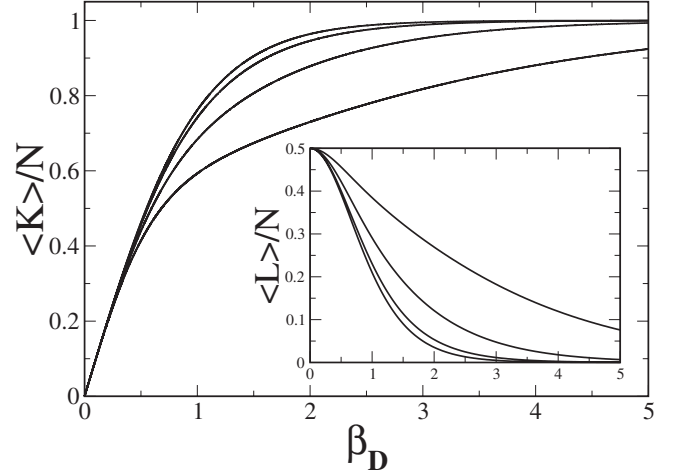


FIG. 1. Averaged complementarity $\frac{1}{N}\langle K \rangle$ as a function of β_D for $\varepsilon=2$. The energy parameter γ takes the values 0, 0.5, 1, and 1.5 (from the left to the right). Inset: normalized number of cavities $\frac{1}{N}\langle L \rangle$ as a function of β_D for the same parameters (γ increases from the left to the right).

of the target's structure and shows also that the number of cavities increases with increasing γ (compare Fig. 1). This has been expected because for larger γ there can appear favorable contributions $-\gamma$ which first of all require the existence of a sufficient number of cavities.

For the analysis of the difference in the free energy of the interaction with the target and rival, we introduce the function

$$B(\varepsilon, \gamma; \beta) := 2 + \frac{1}{\beta \varepsilon} \ln \left(\frac{1 + \exp(-\beta \varepsilon) \cosh(\beta \gamma)}{1 + \exp(\beta \varepsilon) \cosh(\beta \gamma)} \right) \quad (9)$$

and obtain the simple result

$$\langle \Delta F \rangle = -\frac{\varepsilon}{2N} \langle K \rangle (\varepsilon, \gamma; \beta_D) B(\varepsilon, \gamma; \beta) \quad (10)$$

for the selectivity averaged over all possible target and rival structures. Note that $\frac{1}{N}\langle K \rangle(\varepsilon, \gamma=0; \beta_D) = \tanh\left(\frac{\beta_D \varepsilon}{2}\right)$ and $B(\varepsilon, \gamma=0; \beta) = 1$. We compare the characteristic selectivity $\langle \Delta F \rangle$ of this model with the unmodified case $\gamma=0$ and realize that the selectivity decreases for increasing values of γ as shown in Fig. 2. To get a rough estimate of this reduction consider typical values of the interaction parameters. We assume a high degree of optimization during the design step and hence choose β_D to be typically larger than β . For the selectivity shown in Fig. 2 we consider the parameters $\varepsilon=2$, $\beta=0.5$, and $\beta_D=1$ and find that the selectivity is then reduced by 15% for $\gamma=1$.

The burial of solvent molecules as modeled according to Eq. (5) thus does not lead to an enhancement of the selectivity. The primary reason for this is the thermally fluctuating number of cavities so that for increasing γ the system tends to exhibit a larger number of cavities so that beneficial direct contacts are reduced in the contribution to the selectivity. Only energy contributions of direct contacts, however, can discriminate between the differences in the structures of the recognition sites of the target and the rival. The energy con-

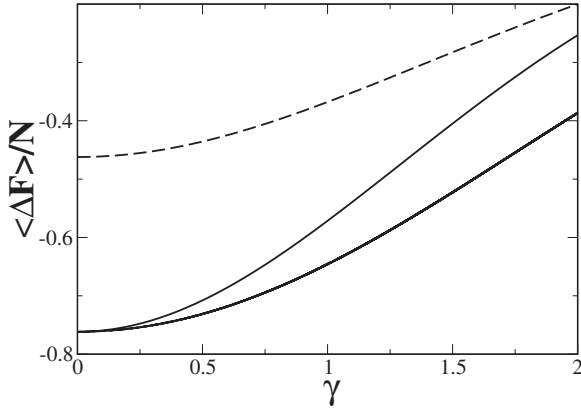


FIG. 2. The averaged selectivity (10) as function of γ for $\varepsilon=2$ with $\beta=1$, $\beta_D=1$ (upper curve) and $\beta=0.5$, $\beta_D=1$ (lower curve). The dashed curve corresponds to the parameters $\beta=1$, $\beta_D=0.5$. An increasing strength of the interaction with the water molecules leads to a reduced selectivity.

tributions from embedded solvent molecules are insensitive to those differences. For large γ the free energy for the interaction of the probe with the rival becomes more similar to the one from the interaction with the target and hence selectivity is reduced. We will come back to this point at the end of Sec. IV A.

IV. OPTIMAL HYDRATION OF GEOMETRIC CAVITIES

In contrast to the previous model, we will now consider protein interfaces where the number of cavities is an intrinsic geometric constraint. Cavities appear in the interface as the roughness of the surface of the proteins might prevent a perfect fit of the shapes of the two proteins at some positions of the interface. For rigid proteins the roughness cannot relax and thus one expects the appearance of a certain number of cavities irrespective of thermal fluctuations. Technically, the number of cavities is controlled by a Lagrange multiplier in our model. So the structure of the molecule is specified not only by the distribution of amino acids but in addition by a Lagrange parameter that contains information about the geometry of the cavities. In addition we allow the cavities to not necessarily be occupied with water molecules, i.e., a single cavity can, but does not have to be filled by solvent. We want to answer the question whether or not there exists a characteristic fraction of occupied cavities which leads to the maximum selectivity in the recognition process. This enables to distinguish between wet and dry interfaces as presented in [9,16,20].

We want to consider a situation where the embedded water molecules mediate interactions between the adjacent amino acids. We therefore require the interaction of a water molecule to depend on the polarity or hydrophobicity of the adjacent monomers and therefore introduce three different energy parameters $\gamma_{PP} > \gamma_{HP} > \gamma_{HH}$. Here the parameter γ_{PP} specifies the strength of the water-bridged interaction in a cavity with two adjacent polar residues (PP cavity), the parameters γ_{HP} and γ_{HH} correspondingly the strength for HP and HH cavities. The order of these parameters reflects the

fact that water itself is polar and therefore the interaction with polar residues is more favorable. Besides, the new parameters have to be chosen in such a way that the interaction strength of direct contacts ε stays larger. The energetics of the mediated interactions is intended to mimic hydrogen bonds between the amino acids that are bridged by solvent molecules. Note that in real interfaces these bridged hydrogen bonds can involve more than one water molecule [18,19]. We will, however, only distinguish between filled and empty cavities, irrespective of the number of contained water molecules.

Let us now define the N -dimensional vector $f = (f_1, \dots, f_N)$, whose i th component specifies whether a cavity at site i is filled by a water molecule ($f_i=1$) or not ($f_i=0$). As we want to consider interfaces with a fixed total number of cavities we adjust this number by a Lagrange parameter μ . In addition we consider the selectivity for varying numbers of embedded water molecules and thus control the number of filled cavities technically by an additional Lagrange parameter ξ . With the use of the abbreviations $\alpha := \gamma_{PP} - \gamma_{HP}$, $\omega := \gamma_{HH} - \gamma_{HP}$ and $\eta := \gamma_{HP} + \xi$ the additional terms in the Hamiltonian that are related to the cavities are then given by

$$\mathcal{H}_{\text{cav}} = - \sum_{i=1}^N \frac{1 - S_i}{2} f_i [\alpha \delta_{\sigma_i, -1} \delta_{\theta_i, -1} + \omega \delta_{\sigma_i, 1} \delta_{\theta_i, 1} + \eta] - \mu \sum_{i=1}^N S_i. \quad (11)$$

The contact variable S_i thus models the appearance of real cavities. Apart from these contributions from solvent in cavities the total energy of the interface contains the usual contact Hamiltonian $\mathcal{H}_{\text{cont}}$ as modeled in Eq. (1) so that $\mathcal{H} = \mathcal{H}_{\text{cont}} + \mathcal{H}_{\text{cav}}$.

The strategy to calculate the selectivity for the above discussed model is outlined in the Appendix. The Lagrange parameters are used to fix the (normalized) number l of cavities in the interface and the fraction f of cavities that are filled with water. We will utilize the normalization that $f \in [0, l]$. We thus obtain the selectivity $\langle \Delta F \rangle_l(f)$ for interfaces with a fixed number of cavities as a function of the number of embedded molecules. We note that the actual results that are presented in the subsequent subsections are obtained with a MATHEMATICA program.

A. Selectivity enhancement

As we want to compare protein interfaces with embedded water molecules, with the dry realization ($f=0$), we consider the correction factor $C_l(f) := \frac{\langle \Delta F \rangle_l(f)}{\langle \Delta F \rangle_l(f=0)}$. The range over f with $C_l(f) > 1$ corresponds to increased selectivity of molecular recognition, whereas a correction factor with $C_l(f) < 1$ describes lowered selectivity due to the inclusion of solvent molecules. Now we are interested in the probability of the macroscopic realization for a wet interface, described by the parameters l and f , in contrast to a dry interface and obtain as a rough estimate

$$\frac{\text{Prob}^{(\text{with water})}}{\text{Prob}^{(\text{dry interface})}} \approx \frac{e^{-\beta \langle \Delta F_l(f) \rangle}}{e^{-\beta \langle \Delta F_l(f=0) \rangle}} \approx e^{N[C_l(f)-1]}. \quad (12)$$

TABLE I. Investigated sets of energy parameters in Eq. (11).

A:	$\varepsilon=2$	$\gamma_{PP}=1$	$\gamma_{HH}=-1$	$\gamma_{HP}=0.5$
B:	$\varepsilon=2$	$\gamma_{PP}=1$	$\gamma_{HH}=-0.5$	$\gamma_{HP}=0.5$
C:	$\varepsilon=2$	$\gamma_{PP}=1$	$\gamma_{HH}=-0.5$	$\gamma_{HP}=0$

To obtain an impression of the size of a possible enhancement of the selectivity due to the inclusion of water we have to choose a characteristic set of the involved parameters. For the discussion we will consider interfaces whose fraction of cavities varies from 10% to 30% ($l=0.1\dots 0.3$) which seems to be reasonable for natural protein-protein interfaces [18,19]. In the following we will discuss the results for $l=0.3$ and note that for $l=0.1$ and $l=0.2$ we obtain qualitatively similar results. Concerning the energy parameters ε , γ_{PP} , γ_{HP} , and γ_{HH} , we will consider exemplarily three different combinations denoted by A, B, and C as shown in Table I. For all combinations of parameters the inclusion of a water molecule in a PP cavity is energetically most favorable, whereas the interaction of a water molecule with at least one polar residue in a HP cavity is more beneficial than in a purely hydrophobic HH cavity. Going from A to B we leave ε , γ_{PP} , and γ_{HP} unchanged, while the change of the parameter γ_{HH} from -1 to -0.5 reduces the penalty for an inclusion of water between two hydrophobic residues. Accordingly, at the change from B to C, the occupation of water between different types of amino acids becomes less favorable. Furthermore, we set $\beta_D=1$ as we want to have a high degree of optimization during the design and $\beta=0.5$, satisfying the relation $\beta\varepsilon=O(1)$.

The correction factor $C_{l=30\%}(f)$ for parameter set A and an interface with 30% cavity area is plotted in Fig. 3 for $N=32$. We were able to show, in general, that the correction factor $C_l(f)$ features a characteristic maximum for some value of f , say f_{opt} , with $C_l(f_{\text{opt}}) > 1$ which is lying somewhere in the allowed interval of f . The existence of a maximum for $C_l(f)$ means that there is a fraction of occupied cavities for which the selectivity of the recognition process becomes maximum. For the considered parameters this typically results in an enhancement of the selectivity for a hydrated interface by a factor of 2–4 using estimate (12) with $N=32$. Note that $N \sim O(30)$ holds for typical interfaces in natural protein-protein complexes [9,10]. The presented example shows that for an interface with 30% cavities roughly one third of the cavities should be filled with water molecules on average to give maximum selectivity. Interestingly, the selectivity first raises up to a maximum with $C_l(f_{\text{opt}}) > 1$ and afterward gets even smaller than one.

We now want to obtain a physical understanding for the evolution of the correction factor $C_l(f)$ which can show both an enhancement and a reduction in the selectivity depending on the degree of hydration of the interface. To this end we consider various observables which characterize the interface between the probe molecule and the target molecule in more detail. The observables provide an answer to the question between which pairs of residues the cavities or the direct contacts are distributed. We define the following quantities which are averaged over the ensemble of probe molecules:

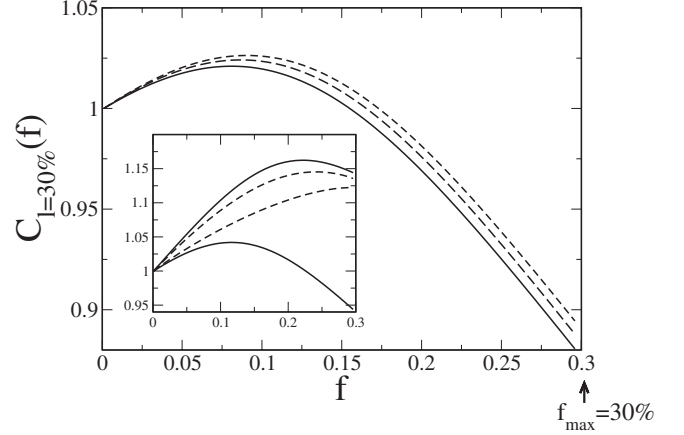


FIG. 3. Analysis of $C_{l=30\%}(f)$ for the parameter set A and $\beta_D=1$, $\beta=0.5$, and $N=32$ [the inset shows parameter set B (lower curve) and C (upper curve), the dashed curve corresponds to set C for $\beta_D=0.8$ and 0.6 from above]. The exactly averaged correction factor is shown together with the approximation discussed in the Appendix ($N=32$ and 64 from above). The maximum at $f_{\text{opt}} \approx 0.085$ leads to an enhancement factor of 1.9 [compare relation (12)]. Note that for the exact average (A7) a value for N has to be specified. Different choices, however, show only very small finite-size variations.

$$W_{n_H^{(T)}}^{\text{PP}} = \sum_{\{\theta\}} W_{\sigma^{(T)}, \theta}^{\text{PP}} P_D(\theta | \sigma^{(T)}), \quad (13)$$

$$W_{n_H^{(T)}}^{\text{HH}} = \sum_{\{\theta\}} W_{\sigma^{(T)}, \theta}^{\text{HH}} P_D(\theta | \sigma^{(T)}), \quad (14)$$

and

$$W_{n_H^{(T)}}^{\text{HP}} = \sum_{\{\theta\}} W_{\sigma^{(T)}, \theta}^{\text{HP}} P_D(\theta | \sigma^{(T)}). \quad (15)$$

These quantities specify how often a particular type of cavity is realized in the interface. We have chosen the index $n_H^{(T)} = N_H^{(T)}/N$ because the obtained expressions only depend on the target's hydrophobicity $N_H^{(T)} = \sum_{i=1}^N \delta_{\sigma_i^{(T)}, 1}$. The formula for $W_{\sigma^{(T)}, \theta}^{\text{PP}}$ is given by

$$W_{\sigma^{(T)}, \theta}^{\text{PP}} := \frac{1}{N} \left\langle \sum_{i=1}^N \frac{1 - S_i}{2} f_i \delta_{\sigma_i^{(T)}, -1} \delta_{\theta_{i-1}} \right\rangle_{\sigma^{(T)}, \theta} \quad (16)$$

for fixed target $\sigma^{(T)}$ and fixed probe molecule θ , similar definitions hold for $W_{\sigma^{(T)}, \theta}^{\text{HH}}$ and $W_{\sigma^{(T)}, \theta}^{\text{HP}}$. The corresponding functions for the direct contacts are indicated by the letter D :

$$D_{n_H^{(T)}}^{\text{PP}} = \sum_{\{\theta\}} D_{\sigma^{(T)}, \theta}^{\text{PP}} P_D(\theta | \sigma^{(T)}), \quad (17)$$

$$D_{n_H^{(T)}}^{\text{HH}} = \sum_{\{\theta\}} D_{\sigma^{(T)}, \theta}^{\text{HH}} P_D(\theta | \sigma^{(T)}), \quad (18)$$

and

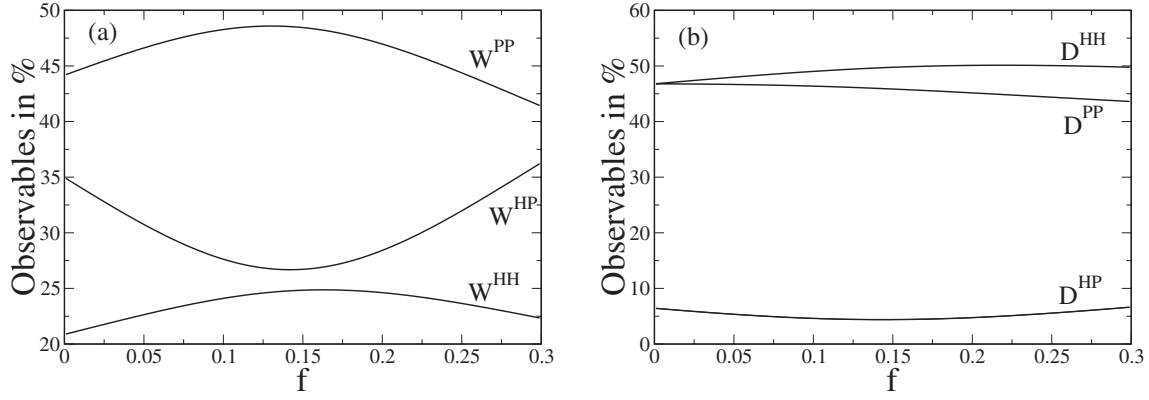


FIG. 4. Analysis of the correction factor $C_{l=30\%}(f)$ (shown in Fig. 3) for the parameter set B and $\beta_D=1$, $\beta=0.5$, and $N=32$. (a) Observables of cavities W^{PP} , W^{HH} , and W^{HP} ; (b) observables of direct contacts D^{PP} , D^{HH} , and D^{HP} . The observables are evaluated for $n_H^{(T)}=0.5$ and are shown in dependence on the normalized f (in %).

$$D_{n_H^{(T)}}^{HP} = \sum_{\{\theta\}} D_{\sigma^{(T),\theta}}^{HP} P_D(\theta|\sigma^{(T)}). \quad (19)$$

The definition of $D_{\sigma^{(T),\theta}}^{PP}$ is analog to the previous functions depending on a fixed target and probe molecule:

$$D_{\sigma^{(T),\theta}}^{PP} := \frac{1}{N} \left\langle \sum_{i=1}^N \frac{1+S_i}{2} \delta_{\sigma_i^{(T)},-1} \delta_{\theta,-1} \right\rangle_{\sigma^{(T),\theta}}. \quad (20)$$

In Fig. 4 we have normalized the given observables to the sum of all direct contacts and to the sum of all occupied cavities respectively. Since the observables have to be computed for a certain hydrophobicity of the target, we have chosen the typical value of $n_H^{(T)}=0.5$. Note that the $n_H^{(T)}=0.5$ terms in Eq. (A7) dominate the sum and hence this also corresponds approximately to an average over all target structures for sufficiently large N (see discussion in the Appendix). For values of $0 \leq f \leq 0.13$ we see that the fraction of favorable embedded water molecules in PP cavities increases. For a small fraction of filled cavities the solvent molecules will preferentially be embedded in PP cavities due to the large energy gain they can provide. This goes along with a weak decrease in direct PP contacts. For a further increasing number of water molecules eventually all PP cavities will be used up and water molecules have to go into the HP cavities as they provide still an energy gain. The relative fraction of occupied PP cavities therefore will be reduced for increasing f . This subsequent decrease in the PP fraction goes along with a decreasing selectivity of the recognition process. We notice that the observables in Fig. 4 take similar values for $f=0$ and $f=f_{\max}=l$ though $C_l(0)=1$ is quite different from $C_l(f_{\max})$ which can even be smaller than one. This demonstrates that the competitive influence of the rival on the selectivity gets more and more important for an increasing number of buried water molecules. Note, however, that the selectivity does not change its sign, so we still have recognition of the target by the probe molecules.

Looking at the observables that describe direct contacts (see Fig. 4), we observe that they show only a weak dependence on f . The fraction of D^{PP} and D^{HH} strongly dominates the direct contacts between different types of amino acids.

For $f>0$ we get $D^{HH} > D^{PP}$ which can be explained in the following way: for an existing site with opposite polar residues (PP) it is more beneficial to fill a cavity with water (in comparison to a HH cavity), and therefore the HH sites are more likely used for direct contacts between the amino acids.

For all results shown in this subsection a high degree of optimization has been assumed ($\beta_D=1$ in comparison to $\beta=0.5$). If the quality of the design is reduced by decreasing the parameter β_D the observed effect of an enhancement of the selectivity due to the inclusion of water molecules in the interface is still present, but becomes weaker and weaker (see inset of Fig. 3). Even for a situation with $\beta=\beta_D=0.5$ selectivity enhancement due to hydration can appear although we note that this is not the case for all sets of the γ_{HH} , γ_{PP} , and γ_{HP} parameters (namely only for set C of the three considered ones).

As a final comment let us come back to the situation where the appearance of cavities is due to thermal fluctuations and where different to the considerations in Sec. III the energy contributions from embedded water particles now distinguish between the different types of amino acids of the cavities. This is technically incorporated if the Lagrange parameters in Eq. (11) are set to zero and thus the number of cavities and the number of embedded solvent molecules fluctuate. The cavity part of the Hamiltonian reads

$$\mathcal{H}_{\text{cav}} = - \sum_{i=1}^N \frac{1-S_i}{2} f_i \Gamma [\alpha \delta_{\sigma_i,-1} \delta_{\theta,-1} + \omega \delta_{\sigma_i,1} \delta_{\theta,1} + \gamma_{HP}] \quad (21)$$

where the parameter Γ specifies the relative weight of the direct contacts and the water-mediated interactions. Notice that different to Eq. (5) no distinction between a favorable and an unfavorable energy contribution of an embedded water molecule is incorporated. The selectivity as a function of the parameter Γ is shown in Fig. 5 (compare also Fig. 2). One finds that the inclusion of water molecules might lead to an enhanced selectivity although an enhancement of the selectivity is not observed for all considered parameter sets. So the distinction of the type of amino acids which are participating in water-mediated interactions is crucial for the ap-

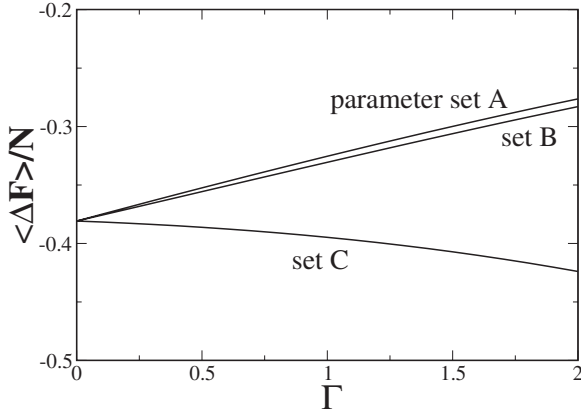


FIG. 5. The averaged selectivity for model (21) with a thermally fluctuating number of cavities as function of Γ for $\beta=0.5$, $\beta_D=1$ and the different relative adjustments of the parameters γ_{PP} , γ_{HH} , and γ_{HP} as specified in Table I.

pearance of an enhanced selectivity due to hydration. We also conclude that the appearance of rigid cavities seems to facilitate the enhancement of selectivity.

B. Dry and wet interfaces

The last part of our investigation examines the influence of the hydrophobicity of the interface on the enhancement of the selectivity of the recognition process. In the previous subsection an average over all possible hydrophobicities has been carried out so that the discussed results are general statements formulated for all classes of proteins and can be understood as a characteristic property of the considered model for molecular recognition. In nature, however, the hydrophobicity is typically different for proteins that fulfill different biological tasks. For example, the average hydrophobicity of the interface of antigen-antibody complexes is relatively small (comparable to the rest of the surface of the protein that is exposed to bulk water) whereas the interfaces of enzyme-inhibitor complexes are largely hydrophobic [7,9,10]. For this reason, we are also interested in an analysis of the free-energy difference for a given class of proteins with fixed (averaged) hydrophobicity $\langle n_H^{(T)} \rangle$ of the target [see the Appendix for the details how the corresponding correction factor $C_l(\langle n_H^{(T)} \rangle; f)$ is evaluated].

We get the result that the correction factor $C_l(\langle n_H^{(T)} \rangle; f)$ for given averaged hydrophobicity $\langle n_H^{(T)} \rangle$ of the target molecules develops a characteristic maximum with $C_l(\langle n_H^{(T)} \rangle) > 1$ for small hydrophobicities so that the selectivity is remarkably enhanced in comparison to the complex with a dry interface (see Fig. 6). For a protein-protein complex with a given small hydrophobicity of the interface the scenario of a dry interface is thus less favorable than the scenario with a hydrated interface. The position f_{opt} of the optimum filling fraction for the class of proteins with a fixed hydrophobicity demands a shift from wet to dry interfaces when the hydrophobicity is increased as shown in Fig. 7. We note, however, that for complexes with large hydrophobicities the recognition is still selective. One also observes that the transition between an optimal dry and wet interface depends on the

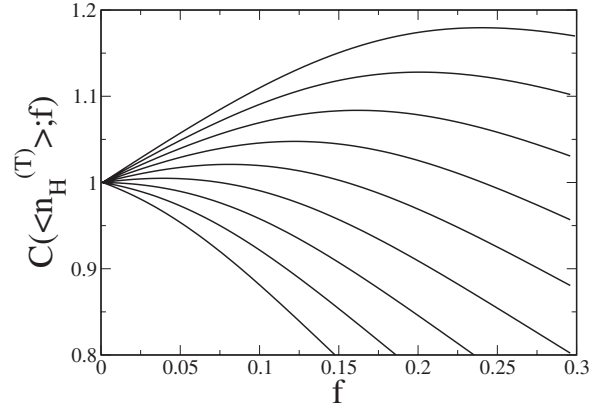


FIG. 6. Correction factor $C_l(\langle n_H^{(T)} \rangle; f)$ as a function of the fraction f of occupied cavities for different fixed hydrophobicities $\langle n_H^{(T)} \rangle$ ranging from 0.1 to 0.9 in units of 0.1 from top to bottom (parameter set A and $l=0.3$).

chosen parameter values for the coupling constants. Our findings thus reproduce the empirically found correlation that the degree of hydration at protein-protein interfaces decreases with the hydrophobicity of the interface (compare [9,16,20]).

We conclude this subsection by considering the modification of model (11) with no discrimination of the type of cavity with respect to the energy gain when water is embedded. In terms of the coupling parameters this means that we have $\gamma_{PP} = \gamma_{HP} = \gamma_{HH} = \gamma$. Again we observe the appearance of a characteristic optimum fraction of occupied cavities which maximizes the selectivity such that $C_l(\langle n_H^{(T)} \rangle; f_{\text{opt}}) > C_l(\langle n_H^{(T)} \rangle; f=0) = 1$. However, if we again consider interfaces with a varying hydrophobicity $\langle n_H^{(T)} \rangle$ of the target the position f_{opt} of the selectivity maximum is not shifted as can be understood from the fact that the energy gain due to embedded water molecules cannot resolve the hydrophobicity of the interface. Consequently no transition from a dry to a wet interface shows up for this modification of the cavity Hamiltonian.

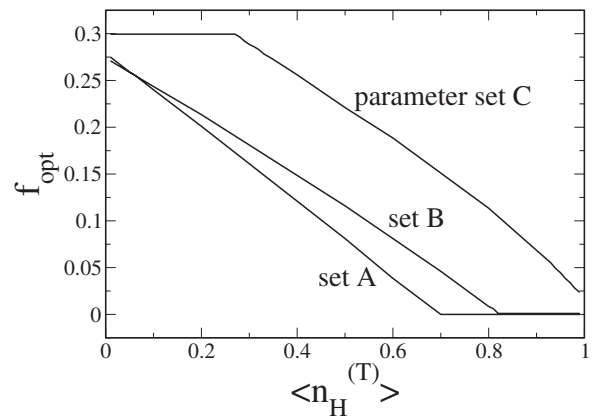


FIG. 7. Position f_{opt} of the selectivity maximum as a function of the hydrophobicity $\langle n_H^{(T)} \rangle$ of the interface with 30% cavities. $f_{\text{opt}} = 0$ favors a dry interface; $f_{\text{opt}} = 0.3$ corresponds to a maximally hydrated (wet) protein interface.

V. SUMMARY

On the basis of coarse-grained modeling we have investigated the influence of solvent molecules on molecular recognition and found that they can provide an enhanced selectivity. To describe the molecular recognition, we have adopted a two-stage approach containing a design of probe molecules and a testing of their recognition ability. The energy that stabilizes the protein-protein complex is described in a coarse-grained view on the level of the hydrophobicity of the amino acids and the residual solvent molecules embedded at the interface.

We discussed a model with an inclusion of water molecules in every cavity at the interface without any coupling to the composition of residues of the two proteins. For all kinds of additional interaction strengths the selectivity of the recognition process is then decreased. The focus of our investigation was then set on the model with an optional inclusion of water molecules at the interface. Additionally the interaction of water depends on the adjacent types of monomers. Having fixed the average number of cavities at the proteins' interface as an intrinsic geometric constraint we have found that there is a characteristic fraction of occupied cavities such that the selectivity becomes maximum. We showed that in many cases it is advantageous to have an occupied fraction in between 25% and 75%. The probability to have recognition of the correct target molecule is then typically enhanced by a factor of 2–4. In addition we could establish a correlation between the degree of hydration of the interface and its hydrophobicity which naturally leads to a discrimination of dry and wet interfaces. We thus reproduce empirical findings for real protein-protein interfaces on the level of a coarse-grained model. We finally conclude that embedded solvent molecules have to be considered as an active part of molecular recognition processes and can considerably contribute to the selectivity.

ACKNOWLEDGMENTS

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APPENDIX: EVALUATION OF THE SELECTIVITY

In this rather technical appendix we outline the strategy to evaluate the selectivity of the recognition process where a specified fraction of cavities is filled with water molecules. The energy contributions at the interface are modeled by Hamiltonian (11).

Following the two-step-approach to obtain the selectivity, we first calculate the conditional probability $P_D(\theta|\sigma^{(T)}) = \frac{1}{Z_D} \sum_{\{S\}} \sum_{\{f\}} \exp\{-\beta_D \mathcal{H}(\sigma^{(T)}, \theta; S, f)\}$ in the design step. We emphasize that the Lagrange parameters $\mu = \mu(\sigma^{(T)}, \theta; \beta_D)$ and $\xi = \xi(\sigma^{(T)}, \theta; \beta_D)$ that have to be used for the design both depend on the structure of the target $\sigma^{(T)}$ and a certain probe molecule θ and the design conditions β_D . For each interaction of the probe with a molecule (target or rival) a different set of Lagrange parameters has to be specified in the most general situation. At the transition to the testing step, we

consequently need to introduce additional sets of different Lagrange multipliers corresponding to the interaction of the probe with both the target $\sigma^{(T)}$ and the rival molecule $\sigma^{(R)}$ at inverse temperature β . However, this most general treatment is rather cumbersome. Instead, we fix the number of given cavities and the fraction of the occupied cavities at the design step and attribute this adjustment as an intrinsic geometric property to the probe molecules which is conserved at the testing step. In doing so only one set of Lagrange parameters is necessary in the testing step. This set is determined in the design and exhibits a dependence on the previously fixed structure of the target. The structure of the probe molecule at the interface is thus specified by the set $(\theta, \mu(\sigma^{(T)}, \theta), \xi(\sigma^{(T)}, \theta))$. The cavity part of the Hamiltonian for the testing step is hence given by Eq. (11) with the set of Lagrange parameters obtained in the design step.

Before we can evaluate the free-energy difference we have to calculate the Lagrange multipliers μ and ξ . Since the fixing of the expectation values of the normalized number of cavities l and the fraction of occupied cavities f suffices to be softly implemented for the ensemble of probe molecules, we just regard the averaged quantities $l_{\sigma^{(T)}} = \sum_{\{\theta\}} l_{\sigma^{(T)}, \theta} P_D(\theta|\sigma^{(T)})$ and $f_{\sigma^{(T)}} = \sum_{\{\theta\}} f_{\sigma^{(T)}, \theta} P_D(\theta|\sigma^{(T)})$ where

$$l_{\sigma^{(T)}, \theta} = \frac{1}{N} \sum_{i=1}^N \left\langle \frac{1 - S_i}{2} \right\rangle_{\sigma^{(T)}, \theta} \tag{A1}$$

and

$$f_{\sigma^{(T)}, \theta} = \frac{1}{N} \sum_{i=1}^N \left\langle \frac{1 - S_i}{2} f_i \right\rangle_{\sigma^{(T)}, \theta} \tag{A2}$$

denote thermal averages in the design step (including the Lagrange parameters) with fixed $\sigma^{(T)}$ and θ . One can show that the analytically obtained results for $l_{\sigma^{(T)}}$ and $f_{\sigma^{(T)}}$ do not depend on the exact structure of $\sigma^{(T)}$ but only on the target's hydrophobicity $N_H^{(T)}$ given by $N_H^{(T)} = N n_H^{(T)} = \sum_{i=1}^N \delta_{\sigma_i^{(T)}, 1}$.

The free energy turns out to be determined by the structural differences between the recognition sites of the target and the rival. To write the result of the free-energy difference in a compact way we need to define quantities that specify the differences of the target and the rival molecules. We thus define $X = \sum_{i=1}^N \delta_{\sigma_i^{(T)}, 1} \delta_{\sigma_i^{(R)}, -1} \in \{0, \dots, N_H^{(T)}\}$ and $Y = \sum_{i=1}^N \delta_{\sigma_i^{(T)}, -1} \delta_{\sigma_i^{(R)}, 1} \in \{0, \dots, N - N_H^{(T)}\}$. The free-energy difference for a given target and rival structure is then given by

$$\Delta F(\sigma^{(T)}, \sigma^{(R)}) = -\frac{1}{\beta} B(\alpha, \omega) X - \frac{1}{\beta} B(\omega, \alpha) Y, \tag{A3}$$

where we have introduced the function $B(\alpha, \omega)$,

$$B(\alpha, \omega) := \frac{G_D(\varepsilon, \omega) \ln \frac{G(\varepsilon, \omega)}{G(-\varepsilon, 0)} + G_D(-\varepsilon, 0) \ln \frac{G(-\varepsilon, 0)}{G(\varepsilon, \alpha)}}{4e^{2\beta_D \mu} \cosh(\beta_D \varepsilon) + 2 + e^{\beta_D \eta} (1 + e^{\beta_D \omega})} \tag{A4}$$

with

$$G(x, y) = 2e^{\beta x + 2\beta \mu} + 1 + e^{\beta(\eta + y)} \tag{A5}$$

and similarly

$$G_D(x, y) = 2e^{\beta_D x + 2\beta_D \mu} + 1 + e^{\beta_D(\eta + y)}. \quad (\text{A6})$$

Note that the auxiliary function $B(\alpha, \omega)$ implicitly depends on the structure $\sigma^{(T)}$ of the target through the dependency on the Lagrange parameters. As already mentioned above, this dependence is, however, reduced to a dependence on the hydrophobicity $N_H^{(T)}$ of the target, that is $B(\alpha, \omega) = B(\alpha, \omega; N_H^{(T)})$. For this reason, averaging over all possible structures of the target and rival molecules will “only” demand the computation of $O(N)$ terms instead of an explicit evaluation for all 2^N configurations. Using the expressions for $l_{\sigma^{(T)}} = l_{\sigma^{(T)}(N_H^{(T)}; \mu_{N_H^{(T)}}, \xi_{N_H^{(T)}})}$ and $f_{\sigma^{(T)}} = f_{\sigma^{(T)}(N_H^{(T)}; \mu_{N_H^{(T)}}, \xi_{N_H^{(T)}})}$, we can set $l_{\sigma^{(T)}}$ and $f_{\sigma^{(T)}}$ to some desired numbers l and f , respectively, and get numerically the values of $\mu_{N_H^{(T)}}$ and $\xi_{N_H^{(T)}}$.

Instead of computing $\Delta F(\sigma^{(T)}, \sigma^{(R)})$ for a specific configuration of $(\sigma^{(T)}, \sigma^{(R)})$ —or due to the sole dependence on the hydrophobicity, for a given combination $(N_H^{(T)}, N_H^{(R)})$ —we average over the ensemble of probe molecules which leads to the expression $\langle \Delta F \rangle_l(f)$, depending on the fixed (average) number of cavities l and the occupied fraction f . The possible values of f are extrapolated to the real interval $[0, l]$. The expression for $\langle \Delta F \rangle_l(f)$ is given by

$$\langle \Delta F \rangle_l(f) = \sum_{N_H^{(T)}=0}^N S(N_H^{(T)}; l, f) \quad (\text{A7})$$

with

$$S(N_H^{(T)}; l, f) = \sum_{X=0}^{N_H^{(T)}} \sum_{Y=0}^{N-N_H^{(T)}} \Omega(N_H^{(T)}, X, Y) \Delta F(N_H^{(T)}) \quad (\text{A8})$$

and

$$\Delta F(N_H^{(T)}) = -\frac{1}{\beta} [B(\alpha, \omega; N_H^{(T)})X + B(\omega, \alpha; N_H^{(T)})Y]. \quad (\text{A9})$$

For the summation over the macroscopic parameters $N_H^{(T)}$, X and Y the corresponding degeneracy (density)

$$\Omega(N_H^{(T)}, X, Y) = \frac{1}{4^N} \binom{N}{N_H^{(T)}} \binom{N_H^{(T)}}{X} \binom{N-N_H^{(T)}}{Y} \quad (\text{A10})$$

of microscopic configurations $\sigma^{(T)}$ with respect to the hydrophobicity $N_H^{(T)}$ has to be taken into account. Using selectivity (A7) we consider the correction factor $C_l(f) := \frac{\langle \Delta F \rangle_l(f)}{\langle \Delta F \rangle_l(f=0)}$ which relates the probability for having a hydrated interface

with $f \neq 0$ to the one for a dry interface with $f=0$ (see Sec. IV A).

For sufficiently large N a good approximation for $\langle \Delta F \rangle_l(f)$ can be obtained if we estimate the sums in Eq. (A7) by evaluating the strongly peaked function $\Omega(N_H^{(T)}, X, Y)$ at its maximum $\Omega(\frac{N}{2}, \frac{N}{4}, \frac{N}{4})$. This fact may facilitate future calculations, since in the presented context ($N \approx 30, \dots, 60$) there is almost no difference between the exact and the approximated results. In Fig. 3 the correction factor $C_l(f)$ which is discussed in Sec. IV A is shown for the exact average together with the approximation.

The selectivity (A7) involves an average over all target structures which are equally likely (expressed in terms of an average over the corresponding hydrophobicities). For the investigation of the optimal degree of hydration we are also interested in an analysis of the free-energy difference for a given fixed (averaged) hydrophobicity $\langle n_H^{(T)} \rangle$ of the target (see Sec. IV B). To this end an additional Lagrange multiplier ζ that controls the hydrophobicity of the target molecules has to be introduced. Note that similarly the hydrophobicity of the rival has to be fixed by a Lagrange parameter. As long as we choose the target and the rival to have the same hydrophobicity, however, the results discussed below will not depend on the hydrophobicity $N_H^{(R)}$ of the rival. Hence, we replace the probability

$$\propto \binom{N}{N_H^{(T)}}$$

for a configuration to have the hydrophobicity $N_H^{(T)}$ by the modified probability

$$P_\zeta(N_H^{(T)}) = \frac{\exp(-\zeta N_H^{(T)})}{[1 + \exp(-\zeta)]^N} \binom{N}{N_H^{(T)}}. \quad (\text{A11})$$

which can be used to express ζ in terms of a given $\langle n_H^{(T)} \rangle$. Using this probability finally leads to a modified correction factor $C_l(\langle n_H^{(T)} \rangle; f)$ for given averaged hydrophobicity $\langle n_H^{(T)} \rangle$ of the target molecules:

$$C_l(\langle n_H^{(T)} \rangle; f) = \frac{\sum_{N_H^{(T)}=0}^N \left(\frac{\langle n_H^{(T)} \rangle}{1 - \langle n_H^{(T)} \rangle} \right)^{N_H^{(T)}} S(N_H^{(T)}; l, f)}{\sum_{N_H^{(T)}=0}^N \left(\frac{\langle n_H^{(T)} \rangle}{1 - \langle n_H^{(T)} \rangle} \right)^{N_H^{(T)}} S(N_H^{(T)}; l, f=0)}, \quad (\text{A12})$$

where $S(N_H^{(T)}; l, f)$ is function (A8) of $N_H^{(T)}$ and (l, f) .

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