

## Molecular simulation of adsorption from dilute solutions\*

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Adsorption of biomolecules on surfaces is a perennial and general challenge relevant to many fields in biotechnology. A change of the Helmholtz free energy  $\Delta A$  takes place when a molecule becomes adsorbed out of a bulk solution. The purpose of our investigations is to explore routes for the calculation of  $\Delta A$  by molecular simulations.  $\Delta A$  can be obtained both by integration over the mean force on a molecule and *via* the local density. It turns out that the route *via* the potential of mean force prevails over the latter due to better consistency. In this work we present results for systems of 1-centre and 2-centre Lennard-Jones mixtures at a 9/3 Lennard-Jones wall.

**Keywords:** molecular simulation, adsorption, potential of mean force, dilute solutions, change of free energy

Adsorption at solid surfaces is of growing practical importance, e.g. in separation technology, industrial catalysis, and pollution control (Rouquerol *et al.*, 1999). It plays a leading part in the purification of proteins by adsorption chromatography and is also involved in the detection of antigens or antibodies by immunochemical techniques, i.e. ELISA. As a molecule approaches the solid surface, a balance is established between the intermolecular attractive and repulsive forces. If other molecules are already adsorbed, both adsorbent–adsorbate and adsorbate–adsorbate interactions come into play. It is at once evident that description of the adsorption is more complicated in the case of a multicomponent system – especially if the adsorption is taking place from a solution at a liquid–solid interface (Rouquerol *et al.*, 1999).

Over the last several years there has been significant interest in using molecular simulations to understand the behaviour of adsorbed solutions on solid surfaces (Monson, 1987; Maddox & Rowlinson, 1993). Much of this interest has come from a need to understand the molecular basis of adsorption separations (Vuong & Monson, 1999). By molecular simulation one can yield structural and thermodynamic information about a system.

A thermodynamic quantity of particular interest is the change of the Helmholtz free energy  $\Delta A$  during adsorption of a solute molecule out of a bulk solution. The most favourite position, orientation, and configuration of the adsorbed molecule will be determined by the minimum of the free energy (Fig. 1). In general,  $\Delta A$  is given by the potential of mean force (PMF) which was introduced by Kirkwood (1935). The PMF was used recently among others for the minimisation of the free energy of isolated proteins by Sippl (1990). For the first time the PMF was used in molecular simulations for the calculation of the change of the free energy during adsorption by Billes *et al.* (2003). The PMF can be obtained *via* two different routes – first by a path integral over the mean force (MF) on the solute molecule, and second over the local density.

After deriving the working equations we performed molecular dynamics simulations for pure Lennard-Jones (LJ) fluids and several dilute LJ mixtures in contact with a plane LJ 9/3 wall in order to explore both routes for the calculation of  $\Delta A$ . From the results for these simple systems we want to figure out which route is more appropriate for an extension to complex molecules like proteins.

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**Abbreviations:**  $\Delta A$ , change of the Helmholtz free energy;  $\Delta W$ , change of the Helmholtz free energy;  $\epsilon$ , potential parameter;  $\langle F_B \rangle$  mean force on particle B; LJ, Lennard-Jones;  $L_{x,y,z}$  box length in x-, y-, z-direction; MF, mean force;  $n$ , local density;  $p$ , protein; PMF, potential of mean force;  $s$ , solid surface;  $\sigma$ , size parameter;  $w$ , water molecule.

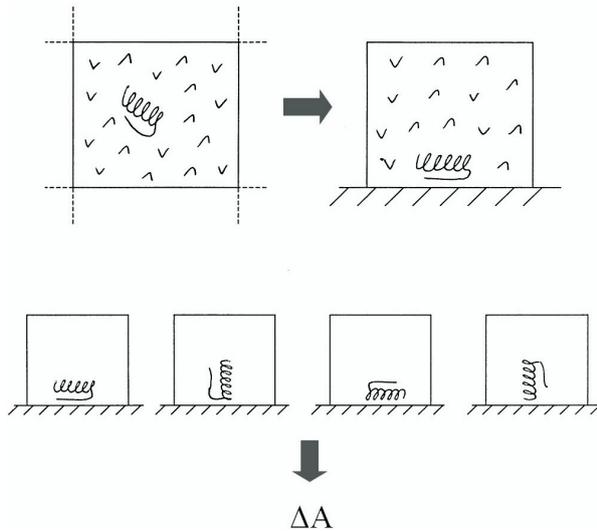


Figure 1. Determination of the most favourite position, orientation and configuration over the Helmholtz free energy  $\Delta A$ .

### DERIVATION OF THE WORKING EQUATIONS

The model (Fig. 2) consists of a rigid solid surface ( $s$ ), and of  $N$  water molecules ( $w$ ) and one protein molecule ( $p$ ) as fluid phase. The protein is, as usually, modelled by several interaction sites. The potential energy of all interactions within the protein in a given configuration shall be denoted by  $U_{pp'}$  and between all water molecules in a given configuration by  $U_{ww}$ .  $U_{pw}$  is the interaction between the water molecules and the protein sites,  $U_{sp}$  the whole interaction of the protein with the solid, and  $U_{sw}$  the sum of all water–solid interactions. Hence, the total potential energy  $U$  of the system is given by  $U = U_{pp} + U_{pw} + U_{ww} + U_{sp} + U_{sw}$ . It may be worth, however, to emphasise already now that the resulting equations can be used for a mixture with an arbitrary number of  $p$  and  $w$  molecules or even a pure fluid of  $w$ . The change of the free energy, however, refers always to the adsorption of just one molecule out of the bulk solution.

### Route of the mean force

Molecule B, simplified as a sphere, is fixed in a given position whilst all A-molecules can move according to the forces exerted on them. In other words, molecule B is considered like an external potential. The item at issue is the change of the free energy  $\Delta A$  during adsorption of the molecule B. If B is fixed at position  $z_1$  we have system 1, position  $z_2$  gives system 2 (Fig. 3) – both with different free energies.  $\Delta A$  is given by  $\Delta A = A(z_1) - A(z_2)$ . In order to obtain  $\Delta A$ , we can form the derivative of  $A$  with respect to the coordinate  $z$  of particle B and perform thereafter an integration over the adsorption path. This gives  $\Delta A = \int (\partial A / \partial z) dz = -\int \langle F_B \rangle dz$ . The con-

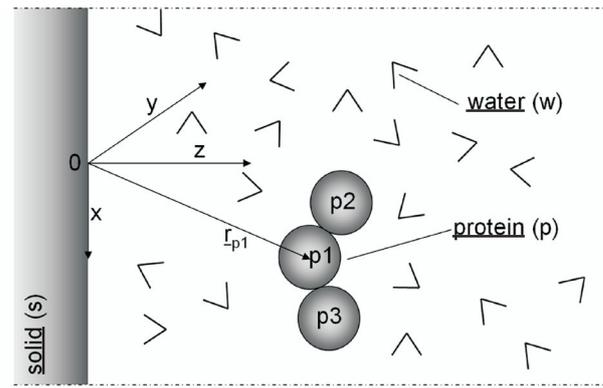


Figure 2. Model for the working equations

tent of this equation is simple and physically appealing. Keeping in mind that  $F_B$  is the force exerted on B, then  $-F_B$  is the reaction force exerted by B. Hence, the integral is mean force times path which is work and the work is the change of the free energy.

### Route of the local density

Here we allow particle B to move freely. A change in the free energy  $W$  is related to the local density  $n_B$  according to  $\Delta W = -kT \Delta \ln n_B(z)$ . We have identified  $\Delta A$  as being a potential of the mean force  $\langle F_B \rangle$ , and according to the derivative of the upper equation (Billes *et al.*, 2003) the same holds for  $\Delta W$ . Thus  $\Delta A = \Delta W$ . From the above equations we see that a second route for the calculation of the change of the free energy during adsorption of a molecule is that *via* the local density.

### Link of both routes

In order to have a clear nomenclature, we will denote by  $\Delta W$  the change in the free energy obtained *via* the local density, and by  $\Delta A$  that obtained *via* integration of the mean force. According to the equations above both values must be equal, which can be expressed by  $\Delta \ln n_B(z) = \beta \int \langle F_B \rangle dz$ , with  $\beta = 1/kT$ . This equation offers the possibility to calculate the local density  $n_B(z)$  *via* the mean force  $\langle F_B \rangle$

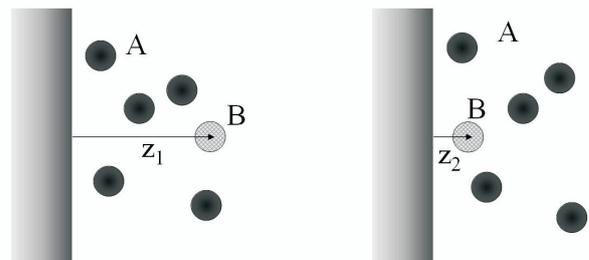


Figure 3. Change of Helmholtz free energy  $\Delta A$  during adsorption for a simple model solution. For explanation see text.

which will turn out to be necessary in the case of a dilute solution.

### SIMULATION RESULTS FOR DIFFERENT SYSTEMS

The working equations above derived will be applied here to a pure Lennard-Jones fluid and several dilute solutions of various particles in contact with a wall in order to explore their practical usefulness. The LJ parameters are denoted as usual by  $\epsilon$  (potential parameter) and  $\sigma$  (size parameter). In the case of a dilute binary mixture, the component in high concentration will be A with LJ parameters  $\epsilon_{AA}$  and  $\sigma_{AA}$  whilst the dilute component is B with  $\epsilon_{BB}$  and  $\sigma_{BB}$ . The interaction between the plane wall and the particles in the fluid is modelled by an LJ 9/3 potential. In the following reduced units will be used.

Molecular dynamics simulations were done using periodic boundary conditions parallel to the surface (in  $x$ - and  $y$ -directions) with a box length  $L_x = L_y$ . The distance between the two LJ walls in  $z$ -direction  $L_z$  is identically equal to  $L_x$  except for case B ( $L_z = 2L_x$ ). The number of A-particles for the simulation systems varied between 1371 (case A), 2743 (case B), and 399 (case C).

For cases A and B the systems were started from a face-centred cubic (fcc) lattice with particles of equal size  $\sigma_{AA}$ . At the beginning of the simulation a fluidisation run was made over 5000 time steps with  $\Delta t = 0.0003$  in reduced units. Thereafter the 9/3 LJ wall was inserted at  $z = -L_x/2$  and moved to  $z = 0$  during another 5000 calculation steps.

For case C the simulation box was filled by randomly distributed and rotated molecules one by one at a lower density (50 kg/m<sup>3</sup>). The production runs (10000 cycles) were started after 2250 equilibration steps by increasing the density up to its end value.

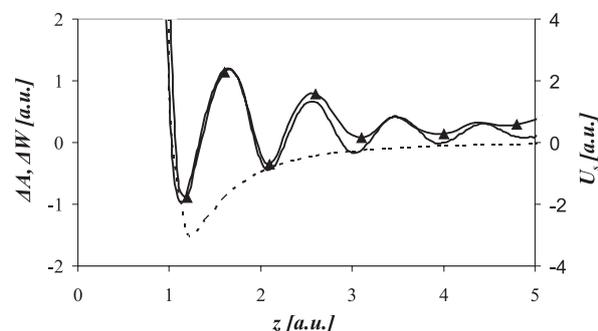
#### Pure LJ-fluid in contact with a wall

A pure LJ fluid in contact with an LJ 9/3 wall is considered. The fluid parameters are  $\epsilon_{AA}$  and  $\sigma_{AA}$  and wall-fluid parameters  $\epsilon_{sA} = 3\epsilon_{AA}$  and  $\sigma_{sA} = \sigma_{AA}$ . First we simply calculated the local density at constant bulk density  $n_b = 0.84$  and temperature  $T = 1.0$  by allowing all particles to move freely. In the second simulation we have fixed one of the LJ particles (particle 1) at a given distance to the wall and calculated the MF. These second simulations were performed for several distances  $z$  of particle 1 from the wall. In Fig. 4 we compare  $\Delta A$  with  $\Delta W$  obtained *via* the local density  $n(z)$ . The conclusion is that the results for the PMF calculated *via* both routes agree remarkably well.

#### Dilute LJ-mixtures in contact with a wall

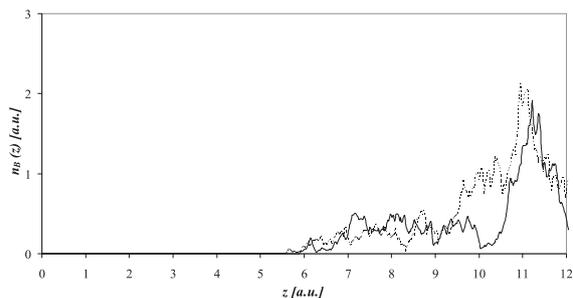
We consider a dilute LJ mixture consisting of one bigger B-particle and an A-particle bulk fluid in contact with an LJ 9/3 wall ( $\sigma_{BB} = 4\sigma_{AA}$ ,  $\epsilon_{sA} = \epsilon_{AA}$  and  $\epsilon_{BB} = 9\epsilon_{AA}$ ; we note that  $\epsilon_{sA} = \epsilon_{AA}$  and the resulting value  $\epsilon_{sB} = 3\epsilon_{AA}$  mean weak attraction from the wall) at constant bulk density  $n_b = 0.70$  and temperature  $T = 1.0$ . First a simulation was performed in order to obtain the local density for component B,  $n_B(z)$ . Results from two simulation runs of different lengths and starting points are displayed in Fig. 5. Next, we calculated the MF on particle B in different fixed positions and obtained the local density over the path integral. We see that the directly calculated  $n_B(z)$  shows strong scattering and little agreement with the density profile obtained *via* the MF. The solute particle stays somewhat remote from the wall. The small circles on the left side indicate A-particles densely packed close to the wall. The big circles indicate the B-particle in two different positions. The bold big sphere at the right side is centred at the maximum of the local density  $n_B(z)$ . There the B-particle is most likely found. The big dashed circle at the left side marks the intuitively given position of the B-particle. However, the fact that the maximum of  $n_B(z)$  appears between 8 and 9 units away from the wall can be explained by the formation of very dense layers of A-molecules close to the wall into which the B particle cannot penetrate. On the other hand, the wall and the dense A-layers exert a certain attraction on B such that its favourite position is somewhat remote from the wall but not in the bulk of the liquid.

The differing size of particle B plays an important role in achieving the PMF and consequently its density profile. Increasing diameters induce global density maxima at even larger distances to the wall provoked by a dense layer of solvent particles between the adsorbing surface and the solute mole-



**Figure 4. Potential of mean force (PMF) results for a pure LJ fluid in contact with an LJ 9/3 wall.**

(▲) PMF  $\Delta A$  calculated by integration of  $\langle F_B \rangle$ ; (—) PMF  $\Delta w$  obtained over local density  $n_B(z)$ ; (- - -) wall potential  $U_s$ .

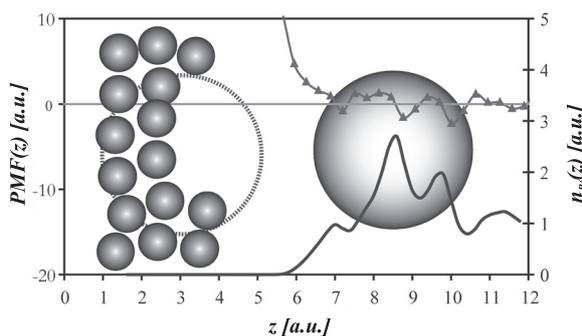


**Figure 5.** Directly calculated local density for a diluted sphere B in contact with a wall ( $\sigma_{BB} = 4\sigma_{AA}$ ,  $\epsilon_{sA} = \epsilon_{AA}$  and  $\epsilon_{BB} = 9\epsilon_{AA}$ ) from initial positions (—)  $z_B = 12.51$  after averaging over  $2.0 \times 10^6$  time steps, and (- - -)  $z_B = 9.39$  after averaging over  $1.6 \times 10^6$  time steps.

cule. One could imagine that for a molecule B of an increasing volume it is getting more and more difficult to penetrate into the area of a higher density of particles A. Subsequently, staying somewhat remote from the wall is a consequence of volume fraction.

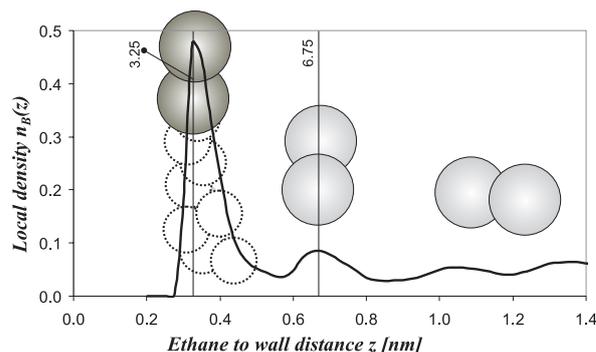
#### Diluted dumbbell in contact with a wall

The system is a binary mixture of a dumbbell B (ethane) diluted in methane A and in contact with a 9/3 LJ graphite wall at a constant density of 500 kg/m<sup>3</sup> and temperature  $T = 160\text{K}$ . The intramolecular potential functions and intermolecular potential parameters refer to the CHARMM force field (MacKerell *et al.*, 1998). Throughout the simulations the centre of mass is kept at a fixed position. Circles on the left side show densely packed A-particles close to the wall (Fig. 7). The dumbbell on the left side represents a B-particle in its most likely conforma-



**Figure 6.** Potential of mean force  $PMF(z)$  ( $\blacktriangle$ ) and local density  $n_B(z)$  (—) of a spherical diluted B-particle for the system of Fig. 5 obtained by integration of the mean force.

Small circles on the left side show rather densely packed A-particles close to the wall. The big circle on the right side represents the B-particle centred at the maximum of the local density. The big circle on the left represents a B-particle located in the minimum of the wall potential where B would be in the absence of the A-particles.



**Figure 7.** Local density  $n_B(z)$  (—) of a dumbbell B (ethane) diluted in spheres (methane) and in contact with an LJ wall (graphite) obtained over integration of the mean force by the fixation of the centre of mass.

Dotted circles on the left side symbolise densely packed A-spheres close to the wall. The dark dumbbell on the left side shows the B-particle in its most preferred conformation in the maximum of the local density. Brighter dumbbells indicate preferred orientations of particle B at further positions in the system.

tion at the maximum of the local density. Obviously the B-particle is attracted by the wall. As expected the dumbbell is parallel-oriented to the surface in the global maximum of the density. Other dumbbells shown in this Figure delineate the orientation of the single B-particle in different distances from the wall. The most preferred orientation of molecule B at the position of the second highest density peak is also parallel to the surface. Furthermore, the more remote B-particles are located in rather arbitrary orientations.

## SUMMARY AND CONCLUSION

We presented two possible routes for the calculation of the change of the free energy during adsorption of a molecule; *via* integration of the mean force on a fixed interaction site or the centre of mass, and over the local density of the solute molecule. Moreover, by combining both routes it is also possible to calculate the local density *via* the mean force. Following that we have explored the applicability of both routes in molecular simulations for pure Lennard-Jones fluids, diluted spheres, and dumbbells. The most interesting finding is that direct simulations do not yield reliable local densities for several dilute solutions, whilst the path integral over the mean force can always be calculated with reasonable accuracy.

Our findings for spherical particles of a volume difference 64:1 and for dumbbells give us hints how the results for PFM and density profile for medium sized biomolecules could look like, although there are two major flaws of these principle studies. Firstly, long range forces and polar behaviour

are not considered in our simulations. Secondly, all the particles are considered to be either spherical or dumbbell-like. Thus, more complex systems would involve not only more realistic geometries, but also structural changes even if simplifications have to be done due to tremendous computation time.

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